

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

57132 (46342)

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

10/070241

INTERNATIONAL APPLICATION NO.
PCT/JP00/05684INTERNATIONAL FILING DATE
24/08/00PRIORITY DATE CLAIMED
27/08/99

TITLE OF INVENTION

NOVEL G PROTEIN-COUPLED RECEPTOR PROTEIN AND DNA THEREOF

APPLICANT(S) FOR DO/EO/US

TAKUYA WATANABE, ET AL.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☒ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ has been communicated by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☒ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

PCT/RO/101; PCT/RO/105; PCT/IB/301; PCT/IB/304; PCT/ISA/202; PCT/ISA/220; PCT/IPEA/401; PCT/IPEA/402;
PCT/IPEA/408; PCT/IPEA/416; PCT/LB/332; PCT/IB/308.

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.101) 10/070241		INTERNATIONAL APPLICATION NO. PCT/JP00/05684		ATTORNEY'S DOCKET NUMBER 57132 (46342)	
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24. The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :				CALCULATIONS PTO USE ONLY	
<input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1040.00					
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00					
<input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$740.00					
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00					
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$740.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).				\$0.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	14 - 20 =	0	x \$18.00	\$0.00	
Independent claims	1 - 3 =	0	x \$84.00	\$0.00	
Multiple Dependent Claims (check if applicable).				<input checked="" type="checkbox"/> \$280.00	
TOTAL OF ABOVE CALCULATIONS =				\$1,020.00	
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				\$0.00	
SUBTOTAL =				\$1,020.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).				\$0.00	
TOTAL NATIONAL FEE =				\$1,020.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).				<input checked="" type="checkbox"/> \$40.00	
TOTAL FEES ENCLOSED =				\$1,060.00	
				Amount to be: refunded	\$
				charged	\$

a. ☒ A check in the amount of **\$1,060.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **04-1105** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

David G. Conlin
Dike, Bronstein, Roberts & Cushman
Intellectual Property Practice Group of
Edwards & Angell, LLP
PO BOX 9169
Boston, MA 02209

Dianne Rees
 SIGNATURE

Dianne Rees, Ph.D.
 NAME

45,281
 REGISTRATION NUMBER

27 February 2002
 DATE

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HARBOR CONSULTING
Intellectual Property Services
1500A Lafayette Road
Suite 262
Portsmouth, N.H.
800-318-3021



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Attorney Docket No. Docket No. 57127 (46342)
#4 Box Set

IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

APPLICANTS Takuya WATANABE, et al. EXAMINER: Not Yet assigned
U.S.S.N.: 10/070,241 GROUP: Not Yet assigned
FILED (I.A.): August 24, 2000 Conf. No. 2935
FOR: Novel G Protein-Coupled Receptor Protein and DNA Thereof

CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service with sufficient postage as Express Mail in an envelope addressed to: BOX MISSING PARTS, Commissioner for Patents, Washington, D.C. 20231 on July 22, 2002, under Express Mail Label No. EV 097 094 034 US

By: Kelen Murray Carl

BOX MISSING PARTS
Commissioner for Patents
Washington, D.C. 20231

Sir/Madam:

RESPONSE TO NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. § 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

The following is in response to the Notification Of Missing Requirements Under 35 U.S.C. § 371 In The United States Designated/Elected Office (DO/EO/US), mailed May 22, 2002, in the above-referenced application.

Enclosed herewith for filing in the subject application are the following:

1. A copy of the Notification Of Missing Requirements;
2. Paper Copy of Sequence Listing;
3. Computer Readable Form (CRF) of Sequence Listing;

Please delete the paragraph on page 11, lines 11-15, and replace it with the following paragraph:

FIG. 2 shows the base sequence of DNA (SEQ ID NO: 2) encoding the human brain-derived protein (SEQ ID NO: 1) (AQ27) of the present invention obtained in Example 1, and the amino acid sequence deduced from the base sequence (continued from Figure 1).

REMARKS

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Date January 31, 2003

By Dianne Rees

EDWARDS & ANGELL, LLP
P.O. Box 9169
Boston, Massachusetts 02209
Telephone: (617) 439-4444
Facsimile: (617) 439-4170

Dianne M. Rees, Ph.D.
Attorney for Applicant
Registration No. 45,281

Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 04-1105 for any such fees; and applicant(s) hereby petition for any needed extension of time.
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Attorney Docket No. 57127 (46342)
U.S.S.N. 10/070,241
Filed (I.A.): August 24, 2000
Page 2 of 3

4. Amendment directing entry of Paper Copy of Sequence Listing into specification [included in this paper];
5. Statement Accompanying Submission Of Sequence Listing Under 37 C.F.R. § 1.821(g) (included in this paper);
6. A return receipt postcard.

AMENDMENT DIRECTING ENTRY OF SEQUENCE LISTING

At the last page of the specification, please enter the attached paper copy of the Sequence Listing (6 pages) and number the pages as appropriate.

STATEMENT ACCOMPANYING SUBMISSION OF SEQUENCE LISTING

Provided herewith is a Paper Copy of a Sequence Listing for the nucleotide and/or amino acid sequence(s) in this application. Each Sequence has been assigned a separate identifier as required in 37 C.F.R. § 1.821(c) and 37 C.F.R. §§ 1.822 and 1.823. An amendment directing entry of the Paper Copy of the Sequence Listing into the specification is provided above.

Applicants further provide a Computer Readable Form (CRF) corresponding to the Paper Copy of the Sequence Listing provided herewith. Pursuant to 37 C.F.R. § 1.821(g), Applicants' agent hereby states that the CRF corresponds exactly to the Paper Copy of the Sequence Listing provided herewith. Applicants' agent further hereby states that the contents of the Paper Copy of the Sequence Listing and CRF do not go beyond the disclosure in the Application as filed and do not introduce new matter.

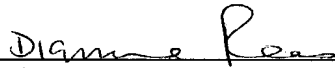
Attorney Docket No. 57127 (46342)
U.S.S.N. 10/070,241
Filed (I.A.): August 24, 2000
Page 3 of 3

If for any reason, a fee paid is inadequate or credit is owed for any excess fee paid, you are hereby authorized and requested to charge Deposit Account No. **04-1105**.

Respectfully submitted,

Date:

July 22, 2002



Dianne M. Rees, Ph.D.
Reg. No. 45,281
EDWARDS & ANGELL, LLP
P.O. Box 9169
Boston, MA 02209

Telephone: 617-439-4444
Customer No. 21874

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P01-0257/2633WOOP

SPECIFICATION**NOVEL G PROTEIN-COUPLED RECEPTOR PROTEIN AND
DNA THEREOF**

5

FIELD OF THE INVENTION

The present invention relates to a human brain-derived novel protein (G protein-coupled receptor protein) or its salt, a DNA encoding the same and the like.

10

BACKGROUND ART

A variety of physiologically active substances such as hormones, neurotransmitters, etc. regulate the functions in vivo through specific receptor proteins located in a cell membrane. Many of these receptor proteins are coupled with guanine nucleotide-binding protein (hereinafter sometimes referred to as G protein) and mediate the intracellular signal transduction via activation of G protein. These receptor proteins possess the common structure, i.e. seven transmembrane domains and are thus collectively referred to as G protein-coupled receptors or seven-transmembrane receptors (7TMR).

G protein-coupled receptor proteins present on the cell surface of each functional cells and organs in the body, and play important physiological roles as the targets of molecules that regulate the functions of the cells and organs, e.g., hormones, neurotransmitters, physiologically active substances and the like.

30

To clarify the relationship between substances that regulate complex biological functions in various cells and organs and their specific receptor proteins, in particular, G protein-coupled receptor proteins, would elucidate the functional mechanisms in various

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cells and organs in the body to provide a very important means for development of drugs closely associated with the functions.

For example, in central nervous system organs such
5 as brain, their physiological functions of brain are controlled in vivo through regulation by many hormones, hormone-like substances, neurotransmitters or physiologically active substances. In particular, physiologically active substances are found in numerous
10 sites of the brain and regulate the physiological functions through their corresponding receptor proteins. However, it is supposed that many unknown hormones, neurotransmitters or other physiologically active substances still exist in the brain and, as for their
15 cDNAs encoding receptor proteins, many of such cDNAs have not yet been reported. In addition, it is still unknown if there are subtypes of known receptor proteins.

It is also very important for development of drugs
20 to clarify the relationship between substances that regulate elaborate functions in brain and their specific receptor proteins. Furthermore, for efficient screening of agonists and antagonists to receptor proteins in development of drugs, it is required to
25 clarify functional mechanisms of receptor protein genes expressed in brain and express the genes in an appropriate expression system.

In recent years, random analysis of cDNA sequences has been actively studied as a means for analyzing
30 genes expressed in vivo. The sequences of cDNA fragments thus obtained have been registered on and published to databases as Expressed Sequence Tag (EST). However, since many ESTs contain sequence information only, it is difficult to deduce their functions from
35 the information.

DISCLOSURE OF THE INVENTION

The present invention provides a human brain-derived novel protein (G protein-coupled receptor
5 protein) , its partial peptide, or their salts, a DNA comprising a DNA encoding said protein or its partial peptide, a recombinant vector containing said DNA, a transformant transformed by said vector, a process for producing said protein or its salt, an antibody to said
10 protein, its partial peptide or their salts, a determination method of a ligand to the protein (G protein-coupled receptor protein), a method for screening a compound or its salt that alters the binding property between a ligand and the protein (G
15 protein-coupled receptor protein), a kit for the screening described above, a compound or its salt that alters the binding property between a ligand and the protein (G protein-coupled receptor protein), which is obtained by the screening method or the screening kit
20 and a pharmaceutical composition comprising a compound or its salt that alters the binding property between a ligand and the protein.

The present inventors have made extensive studies and as a result, succeeded in isolating cDNAs encoding
25 a human brain-derived novel protein (G protein-coupled receptor protein) and in sequencing their full base sequences. When the base sequences were translated into the amino acid sequences, 1 to 7 transmembrane domains were found to be on the hydrophobic plot,
30 verifying that the proteins encoded by these cDNAs are seven-transmembrane type G protein-coupled receptor proteins (Figure 3). The present inventors have continued extensive studies and as a result, have come to accomplish the present invention.

Thus, the present invention provides, for example, the following:

(1) A protein which comprises the same or substantially the same amino acid sequence as the amino acid sequence represented by SEQ ID NO:1, or a salt thereof.

(2) A partial peptide of the protein according to the above (1), or a salt thereof.

(3) A DNA which comprises a DNA encoding the protein according to the above (1).

(4) A DNA according to the above (3) which has a base sequence represented by SEQ ID NO:2.

(5) A recombinant vector which comprises the DNA according to the above (3).

(6) A transformant transformed with the recombinant vector according to the above (5).

(7) A method for producing the protein or a salt thereof, according to the above (1), which comprises culturing said transformant according to the above (6) and producing and accumulating the protein according to the above (1).

(8) An antibody to the protein according to the above (1), the partial peptide according to the above (2), or a salt thereof.

(9) A method for determination of a ligand to the protein or its salt according to the above (1), which comprises using the protein according to the above (1), the partial peptide according to the above (2), or a salt thereof.

(10) A method for screening a compound or its salt that alters the binding property between a ligand and the protein or its salt according to the above (1), which comprises using the protein according to the above (1), the partial peptide according to the above (2), or a salt thereof.

(11) A kit for screening a compound or its salt that alters the binding property between a ligand and the protein or its salt according to the above (1), which comprises the protein according to the above (1),
5 the partial peptide according to the above (2), or a salt thereof.

(12) A compound that alters the binding property between a ligand and the protein or its salt according to the above (1), which is obtainable by using the
10 screening method according to the above (10) or the screening kit according to the above (11).

(13) A pharmaceutical composition which comprises a compound or its salt that alters the binding property between a ligand and the protein or its salt according
15 to the above (1), which is obtainable by using the screening method according to the above (10) or the screening kit according to the above (11).

(14) A DNA which hybridizes to the DNA according to the above (3) under highly stringent conditions.
20 More specifically, the present invention provides, for example, the following:

(15) The protein according to the above (1), wherein the protein is (i) an amino acid sequence represented by SEQ ID NO:1 of which at least 1 or 2
25 (preferably 1 to 30, more preferably 1 to 9 and most preferably several (1 or 2)) amino acids are deleted; (ii) an amino acid sequence represented by SEQ ID NO:1 to which at least 1 or 2 (preferably 1 to 30, more preferably 1 to 10 and most preferably several (1 or
30 2)) amino acids are added; (iii) an amino acid sequence represented by SEQ ID NO:1 into which at least 1 or 2 (preferably 1 to 30, more preferably 1 to 10 and most preferably several (1 or 2)) amino acids are substituted; and (iv) the protein comprising a
35 combination of the above amino acid sequences.

(16) The method for determination of a ligand according to the above (10), which comprises bringing a test compound in contact with the protein or a salt thereof, according to the above (1) or the partial peptide or a salt thereof, according to the above (2).

(17) A method for determination of a ligand according to the above (9), wherein ligand is angiotensin, bombesin, canavanin, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purines, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal polypeptide), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene-related peptide), leukotrienes, pancreastatin, prostaglandins, thromboxane, adenosine, adrenaline, α and β -chemokines (e.g., IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamin, neurotensin, TRH, pancreatic polypeptide or galanin.

(18) The method of screening according to the above (11), in which (i) the case where a ligand is brought in contact with the protein or its salt according to the above (1) or the partial peptide or its salt according to the above (2) is compared with (ii) the case where the ligand and a test compound are brought in contact with the protein or its salt according to the above (1) or the partial peptide or its salt according to the above (2).

(19) A method of screening a compound or its salt that alters the binding property between a ligand and the protein or its salt according to the above (1), which comprises measuring the amounts of a labeled ligand bound to the protein or its salt according to the above (1) or to the partial peptide or its salt

according to the above (2), (i) when the labeled ligand is brought in contact with the protein or its salt according to the above (1) or with the partial peptide or its salt according to the above (2), and (ii) when
5 the labeled ligand and a test compound are brought in contact with the protein or its salt according to the above (1) or with the partial peptide or its salt according to the above (2); and comparing the amounts measured in (i) and (ii).

10 (20) A method of screening a compound or its salt that alters the binding property between a ligand and the protein or its salt according to the above (1), which comprises measuring the amounts of a labeled
15 ligand bound to a cell containing the protein according to the above (1), (i) when the labeled ligand is brought in contact with the cell containing the protein according to the above (1), and (ii) when the labeled ligand and a test compound are brought in contact with the cell containing the protein according to the above
20 (1); and comparing the amounts measured in (i) and (ii).

(21) A method of screening a compound or its salt that alters the binding property between a ligand and the protein or its salt according to the above (1), which comprises measuring the amounts of a labeled
25 ligand bound to a cell membrane fraction containing the protein according to the above (1), (i) when the labeled ligand is brought in contact with the cell membrane fraction, and (ii) when the labeled ligand and a test compound are brought in contact with the cell
30 membrane fraction; and comparing the amounts measured in (i) and (ii).

(22) A method of screening a compound or its salt that alters the binding property between a ligand and the protein or its salt according to the above (1),
35 which comprises measuring the amounts of a labeled

ligand bound to a protein expressed in a cell membrane,
(i) when the labeled ligand is brought in contact with
the protein expressed in a cell membrane of the
transformant according to the above (6) by culturing
5 the transformant and (ii) when the labeled ligand and a
test compound are brought in contact with the protein
expressed in a cell membrane of the transformant
according to the above (6) by culturing the
transformant; and comparing the amounts measured in (i)
10 and (ii).

(23) A method of screening a compound or its salt
that alters the binding property between a ligand and
the protein or its salt according to the above (1),
which comprises measuring the protein-mediated cell
15 stimulating activities, (i) when a compound that
activates the protein or its salt according to the
above (1) is brought in contact with a cell containing
the protein according to the above (1), and (ii) when a
compound that activates the protein or its salt
20 according to the above (1) and a test compound are
brought in contact with a cell containing the protein
according to the above (1); and comparing the
activities measured in (i) and (ii).

(24) A method of screening a compound or its salt
25 that alters the binding property between a ligand and
the protein or its salt according to the above (1),
which comprises measuring the protein-mediated cell
stimulating activities, when a compound that activates
the protein or its salt according to the above (1) is
30 brought in contact with a protein expressed in a cell
membrane of the transformant according to the above (6)
by culturing the transformant, and when the compound
that activates the protein or its salt according to the
above (1) and a test compound are brought in contact
35 with the protein expressed in a cell membrane of the

transformant according to the above (6) by culturing the transformant; and comparing the protein-mediated activities measured in (i) and (ii).

(25) The method of screening according to the above (23) or (24), in which said compound that activates the protein according to the above (1) is angiotensin, bombesin, canavaninoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, an opioid, a purine, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, vasoactive intestinal and related polypeptide (VIP), somatostatin, dopamine, motilin, amylin, bradykinin, calcitonin gene-related peptide (CGRP), a leukotriene, pancreastatin, a prostaglandin, thromboxane, adenosine, adrenaline, an α - and β -chemokine (e.g., IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1- α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptide, or galanin.

(26) A compound or salts that alters the binding property between a ligand and the protein or its salt according to the above (1), which is obtainable by using the screening method according to the above (18) to (25).

(27) A pharmaceutical composition which comprises a compound or a salts that alters the binding property between a ligand and the protein or its salt according to the above (1), which is obtainable by using the screening method according to the above (18) to (25).

(28) A kit for screening, which comprises the cell which comprising the protein according to the above (1).

(29) A kit for screening according to the above (11), which comprises the cell membrane fraction comprising the protein according to the above (1).

(30) The kit for screening according to the above (11), which comprises the protein expressed at the cell membrane of a transformant by culturing the transformant according to the above (6).

5 (31) A compound or salts which alters the binding property between a ligand and the protein or its salt according to the above (1), which is obtainable by using the screening method according to the above (28) to (30).

10 (32) A pharmaceutical composition which comprises a compound or a salts that alters the binding property between a ligand and the protein or its salt according to the above (1), which is obtainable by using the screening method according to the above (28) to (30).

15 (33) A method of quantifying the protein according to the above (1), the partial peptide according to the above (2), or a salt thereof, which comprises contacting the antibody according to the above (8) with the protein according to the above (1), the partial
20 peptide according to the above (2), or a salt thereof.

 (34) A method of quantifying the protein according to the above (1), the partial peptide according to the above (2) or salts thereof in a test fluid, which comprises competitively reacting the antibody according
25 to the above (8) with a test fluid and a labeled protein according to the above (1), the partial peptide according to the above (2) or salts thereof; and measuring the ratios bound to the antibody of the labeled protein according to the above (1), the partial
30 peptide or its salts according to the above (2).

 (35) A method of quantifying the protein according to the above (1), the partial peptide according to the above (2), or salts thereof in a test fluid, which comprises reacting a test fluid simultaneously or
35 sequentially with the antibody according to the above

5 BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 2 shows the base sequence of DNA encoding the human brain-derived protein (AQ27) of the present invention obtained in Example 1, and the amino acid sequence deduced from the base sequence (continued from Figure 1).

FIG. 4 shows the results of the analysis on the distribution of the expression of AQ27 in the tissues, which was performed in Example 2.

BEST MODE OF EMBODIMENT OF THE INVENTION

30 The protein (G protein-coupled receptor protein) of the present invention is the receptor protein comprising the same or substantially the same amino acid sequence as the amino acid sequence [amino acid

sequences in Figure 1 to Figure 2] represented by SEQ ID NO:1 (hereinafter the protein(G protein-coupled receptor protein) are sometimes referred as the protein of the present invention).

5 The protein of present invention may be any protein (G protein-coupled receptor protein) derived from any cells of human and other warm-blooded animals (e.g. guinea pig, rat, mouse, rabbit, swine, sheep, bovine, monkey, etc.) such as splenic cell, nerve cell,
10 glial cell, β cell of pancreas, bone marrow cell, mesangial cell, Langerhans' cell, epidermic cell, epithelial cell, endothelial cell, fibroblast, fibrocyte, myocyte, fat cell, immune cell (e.g., macrophage, T cell, B cell, natural killer cell, mast
15 cell, neutrophil, basophil, eosinophil, monocyte), megakaryocyte, synovial cell, chondrocyte, bone cell, osteoblast, osteoclast, mammary gland cell, hepatocyte, interstitial cell, etc., the corresponding precursor cells, stem cells, cancer cells and hemocyte type cells
20 (e.g., MEL, M1, CTLL-2, HT-2, WEHI-3, HL-60, JOSK-1, K562, ML-1, MOLT-3, MOLT-4, MOLT-10, CCRF-CEM, TALL-1, Jurkat, CCRT-HSB-2, KE-37, SKW-3, HUT-78, HUT-102, H9, U937, THP-1, HEL, JK-1, CMK, KO-812, MEG-01, etc.); or any tissues where such cells are present, such as brain
25 or any of brain regions (e.g., olfactory bulb, amygdaloid nucleus, cerebral basal bulb, hippocampus, thalamus, hypothalamus, subthalamus, cerebral cortex, medulla oblongata, cerebellum, occipital pole, frontal lobe, temporal lobe, putamen, caudate nucleus,
30 corpus callosum, substantia nigra), spinal cord, hypophysis, stomach, pancreas, kidney, liver, gonad, thyroid, gall-bladder, bone marrow, adrenal gland, skin, muscle, lung, gastrointestinal tract (e.g., large intestine and small intestine), blood vessel, heart,
35 thymus, spleen, submandibular gland, peripheral blood,

peripheral hemocyte, prostate, testis, ovary, placenta, uterus, bone, joint, skeletal muscle, (especially, brain and brain region) etc.; the proteins may also be synthetic proteins.

5 The amino acid sequence which has substantially the same amino acid sequence as that represented by SEQ ID NO:1 includes an amino acid sequence having at least about 70% homology, preferably at least about 80% homology, more preferably at least about 90% homology, and most preferably at least about 95% homology, to the amino acid sequence represented by SEQ ID NO:1.

10 Preferred examples of the protein of the present invention, which comprises substantially the same amino acid sequence as that represented by SEQ ID NO: 1 are proteins having substantially the same amino acid sequence as that represented by SEQ ID NO: 1 and having substantially the same activity as that of the amino acid sequence represented by SEQ ID NO: 1.

15 The substantially equivalent activities are, for example, a ligand binding activity, a signal transduction activity, etc. The term "substantially equivalent" is used to mean that the nature of these activities is equivalent. Therefore, it is preferred that these activities such as ligand binding activity, a signal transduction activity, etc. are equivalent in strength (e.g., about 0.5 to about 2 times), and it is allowable that even differences among grades such as the strength of these activities and molecular weight of the protein are present.

20 The activities such as a ligand binding activity, a signal transduction activity or the like can be assayed according to a publicly known method, for example, by means of ligand determination or screening, which will be later described.

The protein of the present invention which can be employed include proteins comprising (i) an amino acid sequence represented by SEQ ID NO:1, of which at least 1 or 2 (preferably 1 to 30, more preferably 1 to 10 and most preferably several (1 or 2)) amino acids are
5 deleted; (ii) an amino acid sequence represented by SEQ ID NO:1, to which at least 1 or 2 (preferably 1 to 30, more preferably 1 to 10 and most preferably several (1 or 2)) amino acids are added; (iii) an amino acid
10 sequence represented by SEQ ID NO:1, in which at least 1 or 2 (preferably 1 to 30, more preferably 1 to 10 and most preferably several (1 or 2)) amino acids are substituted by other amino acids; and (iv) a combination of the above amino acid sequences.

15 Throughout the present specification, the proteins are represented in accordance with the conventional way of describing peptides, that is, the N-terminus (amino terminus) at the left hand and the C-terminus (carboxyl terminus) at the right hand. In the proteins of the
20 present invention including the proteins containing the amino acid sequence shown by SEQ ID NO:1, the C-terminus is usually in the form of a carboxyl group (-COOH) or a carboxylate (-COO⁻) but may be in the form of an amide (-CONH₂) or an ester (-COOR).

25 Examples of the ester group shown by R include a C₁₋₆ alkyl group such as methyl, ethyl, n-propyl, isopropyl, n-butyl, etc.; a C₃₋₈ cycloalkyl group such as cyclopentyl, cyclohexyl, etc.; a C₆₋₁₂ aryl group such as phenyl, α -naphthyl, etc.; an aralkyl having 7
30 to 14 carbon atoms such as a phenyl-C₁₋₂ alkyl group, e.g., benzyl, phenethyl, etc.; an α -naphthyl-C₁₋₂ alkyl group such as α -naphthylmethyl, etc.; and the like. In addition, pivaloyloxymethyl or the like which is used
35 widely as an ester for oral administration may also be used.

Specifically, the partial peptide of the protein of the present invention having the amino acid sequence represented by SEQ ID NO:1 (Figure 3) is a peptide containing the parts, which have been analyzed to be
5 extracellular domains (hydrophilic domains) in the hydrophobic plotting analysis. A peptide containing a hydrophobic domain part can be used as well. In addition, the peptide may contain each domain separately or plural domains together.

10 The partial peptide of the present invention is a peptide having at least 20; preferably at least 50 and more preferably at least 100 amino acids, in the amino acid sequence, which constitutes the protein of the present invention.

15 The substantially the same amino acid sequence includes an amino acid sequence having at least about 50% homology, preferably at least about 70% homology, more preferably at least about 80% homology, much more preferably at least about 90% homology and most
20 preferably at least about 95% homology, to the amino acid sequence represented.

As used herein the term "substantially equivalent activities" refers to the same significance as defined hereinabove. The "substantially equivalent activities"
25 can be assayed by the same method as described above.

In the partial peptide of the present invention, at least 1 or 2 (preferably 1 to 10, more preferably several (1 or 2)) amino acids may be deleted; at least 1 or 2 (preferably 1 to 20, more preferably 1 to 10 and
30 most preferably several (1 or 2)) amino acids may be added; or at least 1 or 2 (preferably 1 to 10, more preferably 1 to 5, further preferably several (1 or 2)), amino acids may be substituted by other amino acids.

In the partial peptide in the protein of the
35 present invention, the C-terminus is usually in the

form of a carboxyl group (-COOH) or a carboxylate (-COO⁻) but may be in the form of an amide (-CONH₂) or an ester (-COOR), as in the protein of the present invention described above.

5 Furthermore, examples of the partial peptide of the present invention include variants of the above peptides, wherein the amino group at the N-terminal methionine residue is protected with a protecting group, those wherein the N-terminal region is cleaved in vivo
10 and the Gln formed is pyroglutaminated, those wherein a substituent on the side chain of an amino acid in the molecule is protected with a suitable protecting group, or conjugated proteins such as glycoproteins having sugar chains, as in the protein of the present
15 invention described above.

As the salts of the protein of the present invention or its partial peptide, physiologically acceptable acid addition salts are particularly preferred. Examples of such salts are salts with
20 inorganic acids (e.g., hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid), salts with organic acids (e.g., acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic
25 acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid) and the like.

The protein of the present invention or salts thereof may be manufactured by a publicly known method used to purify a polypeptide from human or other warm-blooded animal cells or tissues described above.
30 Alternatively, the protein of the present invention or salts thereof may also be manufactured by culturing a transformant containing DNA encoding the protein of the present invention, as will be later described.
35 Furthermore, the protein of the present invention or

salts thereof may also be manufactured by the methods for synthesizing proteins, which will also be described hereinafter, or by modified methods.

Where the protein or salts thereof are
5 manufactured from human or mammalian tissues or cells, human or mammalian tissues or cells are homogenized, then extracted with an acid or the like, and the extract is isolated and purified by a combination of chromatography techniques such as reverse phase
10 chromatography, ion exchange chromatography, and the like.

To synthesize the protein of the present invention, its partial peptide or its salts or amides, commercially available resins that are used for protein
15 synthesis may be used. Examples of such resins include chloromethyl resin, hydroxymethyl resin, benzhydrylamine resin, aminomethyl resin, 4-benzyloxybenzyl alcohol resin, 4-methylbenzhydrylamine resin, PAM resin, 4-hydroxymethylmehtylphenyl
20 acetamidomethyl resin, polyacrylamide resin, 4-(2',4'-dimethoxyphenyl-hydroxymethyl)phenoxy resin, 4-(2',4'-dimethoxyphenyl-Fmoc-aminoethyl) phenoxy resin, etc. Using these resins, amino acids in which α -amino groups and functional groups on the side chains are
25 appropriately protected are condensed on the resin in the order of the sequence of the objective protein according to various condensation methods publicly known in the art. At the end of the reaction, the protein is excised from the resin and at the same time,
30 the protecting groups are removed. Then, intramolecular disulfide bond-forming reaction is performed in a highly diluted solution to obtain the objective protein or amides thereof.

For condensation of the protected amino acids
35 described above, a variety of activation reagents for

protein synthesis may be used, but carbodiimides are particularly preferably employed. Examples of such carbodiimides include DCC, N,N'-diisopropylcarbodiimide, N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide, etc.

5 For activation by these reagents, the protected amino acids in combination with a racemization inhibitor (e.g., HOBT, HOOBT) are added directly to the resin, or the protected amino acids are previously activated in the form of symmetric acid anhydrides, HOBT esters or
10 HOOBT esters, followed by adding the thus activated protected amino acids to the resin.

Solvents suitable for use to activate the protected amino acids or condense with the resin may be chosen from solvents that are known to be usable for
15 protein condensation reactions. Examples of such solvents are acid amides such as N,N-dimethylformamide, N,N-dimethylacetamide, N-methylpyrrolidone, etc.; halogenated hydrocarbons such as methylene chloride, chloroform, etc.; alcohols such as trifluoroethanol,
20 etc.; sulfoxides such as dimethylsulfoxide, etc.; ethers such as pyridine, dioxane, tetrahydrofuran, etc.; nitriles such as acetonitrile, propionitrile, etc.; esters such as methyl acetate, ethyl acetate, etc.; and appropriate mixtures of these solvents. The
25 reaction temperature is appropriately chosen from the range known to be applicable to protein binding reactions and is usually selected in the range of approximately -20°C to 50°C. The activated amino acid derivatives are used generally in an excess of 1.5 to 4
30 times. The condensation is examined using the ninhydrin reaction; when the condensation is insufficient, the condensation can be completed by repeating the condensation reaction without removal of the protecting groups. When the condensation is yet
35 insufficient even after repeating the reaction,

unreacted amino acids are acetylated with acetic anhydride or acetylimidazole to cancel any possible adverse affect on the subsequent reaction.

Examples of the protecting groups used to protect the starting amino groups include Z, Boc, t-pentyloxycarbonyl, isobornyloxycarbonyl, 4-methoxybenzyloxycarbonyl, Cl-Z, Br-Z, adamantyloxycarbonyl, trifluoroacetyl, phthaloyl, formyl, 2-nitrophenylsulphenyl, diphenylphosphinothioyl, Fmoc, etc.

A carboxyl group can be protected by, e.g., alkyl esterification (in the form of linear, branched or cyclic alkyl esters of the alkyl moiety such as methyl, ethyl, propyl, butyl, t-butyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, 2-adamantyl, etc.), aralkyl esterification (e.g., esterification in the form of benzyl ester, 4-nitrobenzyl ester, 4-methoxybenzyl ester, 4-chlorobenzyl ester, benzhydryl ester, etc.), phenacyl esterification, benzyloxycarbonyl hydrazidation, t-butoxycarbonyl hydrazidation, trityl hydrazidation, or the like.

The hydroxyl group of serine can be protected through, for example, its esterification or etherification. Examples of groups appropriately used for the esterification include a lower C₁₋₆ alkanoyl group, such as acetyl group, an aroyl group such as benzoyl group, and a group derived from carbonic acid such as benzyloxycarbonyl group and ethoxycarbonyl group. Examples of a group appropriately used for the etherification include benzyl group, tetrahydropyranyl group, t-butyl group, etc.

Examples of groups for protecting the phenolic hydroxyl group of tyrosine include Bzl, Cl₂-Bzl, 2-nitrobenzyl, Br-Z, t-butyl, etc.

Examples of groups used to protect the imidazole moiety of histidine include Tos, 4-methoxy-2,3,6-trimethylbenzenesulfonyl, DNP, benzyloxymethyl, Bum, Boc, Trt, Fmoc, etc.

5 Examples of the activated carboxyl groups in the starting amino acids include the corresponding acid anhydrides, azides, activated esters (esters with alcohols (e.g., pentachlorophenol, 2,4,5-trichlorophenol, 2,4-dinitrophenol, cyanomethyl alcohol,
10 p-nitrophenol, HONB, N-hydroxysuccimide, N-hydroxyphthalimide, HOBT)). As the activated amino acids in which the amino groups are activated in the starting material, the corresponding phosphoric amides are employed.

15 To eliminate (split off) the protecting groups, there are used catalytic reduction under hydrogen gas flow in the presence of a catalyst such as Pd-black or Pd-carbon; an acid treatment with anhydrous hydrogen fluoride, methanesulfonic acid,
20 trifluoromethanesulfonic acid or trifluoroacetic acid, or a mixture solution of these acids; a treatment with a base such as diisopropylethylamine, triethylamine, piperidine or piperazine; and reduction with sodium in liquid ammonia. The elimination of the protecting group
25 by the acid treatment described above is carried out generally at a temperature of approximately -20°C to 40°C. In the acid treatment, it is efficient to add a cation scavenger such as anisole, phenol, thioanisole, m-cresol, p-cresol, dimethylsulfide, 1,4-butanedithiol
30 or 1,2-ethanedithiol. Furthermore, 2,4-dinitrophenyl group known as the protecting group for the imidazole of histidine is removed by a treatment with thiophenol. Formyl group used as the protecting group of the indole of tryptophan is eliminated by the aforesaid acid
35 treatment in the presence of 1,2-ethanedithiol or 1,4-

butanedithiol, as well as by a treatment with an alkali such as a dilute sodium hydroxide solution and dilute ammonia.

5 Protection of functional groups that should not be involved in the reaction of the starting materials, protecting groups, elimination of the protecting groups and activation of functional groups involved in the reaction may be appropriately selected from publicly known groups and publicly known means.

10 In another method for obtaining the amides of the protein of the present invention, for example, the α -carboxyl group of the carboxy terminal amino acid is first protected by amidation; the peptide (protein) chain is then extended from the amino group side to a
15 desired length. Thereafter, a protein in which only the protecting group of the N-terminal α -amino group has been eliminated from the peptide and a protein in which only the protecting group of the C-terminal carboxyl group has been eliminated are manufactured.
20 The two proteins are condensed in a mixture of the solvents described above. The details of the condensation reaction are the same as described above. After the protected protein obtained by the condensation is purified, all the protecting groups are
25 eliminated by the method described above to give the desired crude protein. This crude protein is purified by various known purification means. Lyophilization of the major fraction gives the amide of the desired protein.

30 To prepare the esterified protein of the present invention, for example, the α -carboxyl group of the carboxy terminal amino acid is condensed with a desired alcohol to prepare the amino acid ester, which is followed by procedure similar to the preparation of the

amidated protein above to give the desired esterified protein.

The partial peptide or salts of the protein of the present invention can be manufactured by publicly known
5 methods for peptide synthesis, or by cleaving the protein of the present invention with an appropriate peptidase. For the methods for peptide synthesis, for example, either solid phase synthesis or liquid phase synthesis may be used. That is, the partial peptide or
10 amino acids that can construct the protein of the present invention are condensed with the remaining part of the partial peptide of the present invention. Where the product contains protecting groups, these protecting groups are removed to give the desired
15 peptide. Publicly known methods for condensation and elimination of the protecting groups are described in 1) - 5) below.

- 1) M. Bodanszky & M.A. Ondetti: Peptide Synthesis,
20 Interscience Publishers, New York (1966)
- 2) Schroeder & Luebke: The Peptide, Academic Press, New York (1965)
- 3) Nobuo Izumiya, et al.: *Peptide Gosei-no-Kiso to Jikken* (Basics and experiments of peptide synthesis),
25 published by Maruzen Co. (1975)
- 4) Haruaki Yajima & Shunpei Sakakibara: *Seikagaku Jikken Koza* (Biochemical Experiment) 1, *Tanpakushitsu no Kagaku* (Chemistry of Proteins) IV, 205 (1977)
- 5) Haruaki Yajima ed.: *Zoku Iyakuhin no Kaihatsu*
30 (A sequel to Development of Pharmaceuticals), Vol. 14, Peptide Synthesis, published by Hirokawa Shoten

After completion of the reaction, the product may be purified and isolated by a combination of
35 conventional purification methods such as solvent

extraction, distillation, column chromatography, liquid chromatography and recrystallization to give the partial peptide of the present invention. When the partial peptide obtained by the above methods is in a free form, the peptide can be converted into an appropriate salt by a publicly known method; when the protein is obtained in a salt form, it can be converted into a free form or a different salt form by a publicly known method.

The DNA encoding the protein of the present invention may be any DNA so long as it contains the base sequence encoding the protein of the present invention described above. Such a DNA may also be any one of genomic DNA, genomic DNA library, cDNA derived from the cells or tissues described above, cDNA library derived from the cells or tissues described above and synthetic DNA.

The vector to be used for the library may be any of bacteriophage, plasmid, cosmid, phagemid and the like. In addition, the DNA can be amplified by reverse transcriptase polymerase chain reaction (hereinafter abbreviated as RT-PCR) with total RNA or mRNA fraction prepared from the above-described cells or tissues.

Specifically, the DNA encoding the protein of the present invention may be any one of, for example, DNA having the base sequence represented by SEQ ID NO:2 or any DNA having a base sequence hybridizable to the base sequence represented by SEQ ID NO:2 under high stringent conditions and encoding a protein which has the activities substantially equivalent to those of the protein of the present invention (e.g., a ligand binding activity, a signal transduction activity, etc.).

Specific examples of the DNA that is hybridizable to the base sequence represented by SEQ ID NO:2 under high stringent conditions include DNA having at least

about 70% homology, preferably at least about 80% homology, more preferably at least about 90% homology and most preferably at least about 95% homology, to the base sequence represented by SEQ ID NO:2.

5 The hybridization can be carried out by publicly known methods or by a modification thereof, for example, according to the method described in Molecular Cloning, 2nd Ed., J. Sambrook et al., Cold Spring Harbor Lab. Press, (1989). A commercially available library may
10 also be used according to the instructions of the attached manufacturer's protocol. The hybridization can be carried out preferably under high stringent conditions.

 The high stringent conditions used herein are, for
15 example, those in a sodium concentration at about 19 mM to about 40 mM, preferably about 19 mM to about 20 mM at a temperature of about 50°C to about 70°C, preferably about 60°C to about 65°C. In particular, hybridization conditions in a sodium concentration at
20 about 19 mM at a temperature of about 65°C are most preferred.

 More specifically, for the DNA encoding the protein having the amino acid sequence represented by SEQ ID NO:1, there may be employed DNA having the base
25 sequence represented by SEQ ID NO:2.

 The nucleotides (oligonucleotide) comprising the base sequence encoding the protein of the present invention or a part of the base sequence complementary to the DNA is used to mean that not only the DNA
30 encoding the partial peptide of the present invention described below but also RNA are embraced.

 According to the present invention, antisense nucleotides (oligonucleotides) that can inhibit replication or expression of the protein of the resent
35 invention can be designed and synthesized based on the

cloned or determined base sequence information of the DNA encoding the protein. Such a (oligo) nucleotide (nucleic acid) is capable of hybridizing with RNA of G protein coupled protein gene to inhibit the synthesis or function of said RNA or capable of modulating the expression of a G protein-coupled receptor protein gene via interaction with G protein coupled protein-associated RNA. (oligo) nucleotides complementary to selected sequences of RNA associated with G protein-coupled receptor protein and (oligo) nucleotides specifically hybridizable with the selected sequences of RNA associated with G protein-coupled protein are useful in modulating or controlling the expression of a G protein coupled protein gene in vivo and in vitro, and in treating or diagnosing disease later described. The term "corresponding" is used to mean homologous to or complementary to a particular sequence of the base sequence or nucleic acid including the gene. The term "corresponding" between nucleotides, base sequences or nucleic acids and peptides (proteins) usually refers to amino acids of a peptide (protein) under the order derived from the sequence of nucleotides (nucleic acids) or their complements. 5' end hairpin loop, 5' end 6-base-pair repeats, 5' end untranslated region, polypeptide translation initiation codon, protein coding region, ORF translation initiation codon, 3' untranslated region, 3' end palindrome region, and 3' end hairpin loop in the G protein-coupled protein gene may be selected as preferred target regions, though any other region may be selected as a target in G protein coupled protein genes.

The relationship between the targeted nucleic acids and the (oligo) nucleotides complementary to at least a part of the target, specifically the relationship between the target and the (oligo)

nucleotides hybridizable to the target, can be denoted to be "antisense". Examples of the antisense (oligo) nucleotides include polydeoxynucleotides containing 2-deoxy-D-ribose, polydeoxynucleotides containing D-ribose, any other type of polynucleotides which are N-glycosides of a purine or pyrimidine base, or other polymers containing non-nucleotide backbones (e.g., protein nucleic acids and synthetic sequence-specific nucleic acid polymers commercially available) or other polymers containing nonstandard linkages (provided that the polymers contain nucleotides having such a configuration that allows base pairing or base stacking, as is found in DNA or RNA), etc. The antisense polynucleotides may be double-stranded DNA, single-stranded DNA, single-stranded RNA or a DNA:RNA hybrid, and may further include unmodified polynucleotides (or unmodified oligonucleotides), those with publicly known types of modifications, for example, those with labels known in the art, those with caps, methylated polynucleotides, those with substitution of one or more naturally occurring nucleotides by their analogue, those with intramolecular modifications of nucleotides such as those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.) and those with charged linkages or sulfur-containing linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those having side chain groups such as proteins (nucleases, nuclease inhibitors, toxins, antibodies, signal peptides, poly-L-lysine, etc.), saccharides (e.g., monosaccharides, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylating agents, those with modified linkages (e.g., α anomeric nucleic acids, etc.), and

the like. Herein the terms "nucleoside", "nucleotide" and "nucleic acid" are used to refer to moieties that contain not only the purine and pyrimidine bases, but also other heterocyclic bases, which have been modified.

5 Such modifications may include methylated purines and pyrimidines, acylated purines and pyrimidines and other heterocyclic rings. Modified nucleotides and modified nucleotides also include modifications on the sugar moiety, wherein, for example, one or more hydroxyl

10 groups may optionally be substituted with a halogen atom(s), an aliphatic group(s), etc., or may be converted into the corresponding functional groups such as ethers, amines, or the like.

The antisense polynucleotide (nucleic acid) of the

15 present invention is RNA, DNA or a modified nucleic acid (RNA, DNA). Specific examples of the modified nucleic acid are, but not limited to, sulfur and thiophosphate derivatives of nucleic acids and those resistant to degradation of polynucleoside amides or

20 oligonucleoside amides. The antisense nucleic acids of the present invention can be modified preferably based on the following design, that is, by increasing the intracellular stability of the antisense nucleic acid, increasing the cellular permeability of the antisense

25 nucleic acid, increasing the affinity of the nucleic acid to the targeted sense strand to a higher level, or minimizing the toxicity, if any, of the antisense nucleic acid.

Many of such modifications are known in the art,

30 as disclosed in J. Kawakami, et al., Pharm. Tech. Japan, Vol. 8, pp. 247, 1992; Vol. 8, pp. 395, 1992; S. T. Crooke, et al. ed., Antisense Research and Applications, CRC Press, 1993; etc.

The antisense nucleic acid of the present

35 invention may contain altered or modified sugars, bases

or linkages. The antisense nucleic acid may also be provided in a specialized form such as liposomes, microspheres, or may be applied to gene therapy, or may be provided in combination with attached moieties. Such
5 attached moieties include polycations such as polylysine that act as charge neutralizers of the phosphate backbone, or hydrophobic moieties such as lipids (e.g., phospholipids, cholesterol, etc.) that enhance the interaction with cell membranes or increase
10 uptake of the nucleic acid. Preferred examples of the lipids to be attached are cholesterol or derivatives thereof (e.g., cholesteryl chloroformate, cholic acid, etc.). These moieties may be attached to the nucleic acid at the 3' or 5' ends thereof and may also be
15 attached thereto through a base, sugar, or intramolecular nucleoside linkage. Other moieties may be capping groups specifically placed at the 3' or 5' ends of the nucleic acid to prevent degradation by nucleases such as exonuclease, RNase, etc. Such capping
20 groups include, but are not limited to, hydroxyl protecting groups known in the art, including glycols such as polyethylene glycol, tetraethylene glycol and the like.

The inhibitory action of the antisense nucleic
25 acid can be examined using the transformant of the present invention, the gene expression system of the present invention in vivo and in vitro, or the translation system of the G protein-coupled receptor protein in vivo and in vitro. The nucleic acid can be
30 applied to cells by a variety of publicly known methods.

The DNA encoding the partial peptide of the present invention may be any DNA so long as it contains the base sequence encoding the partial peptide of the present invention described above. The DNA may also be
35 any of genomic DNA, genomic DNA library, cDNA derived

from the cells and tissues described above, cDNA library derived from the cells and tissues described above and synthetic DNA. The vector to be used for the library may be any of bacteriophage, plasmid, cosmid and phagemid. The DNA may also be directly amplified by reverse transcriptase polymerase chain reaction (hereinafter abbreviated as RT-PCR) using mRNA fraction prepared from the cells and tissues described above.

Specifically, the DNA encoding the partial peptide of the present invention may be any one of, for example, (1) DNA containing a partial base sequence of the DNA having the base sequence represented by SEQ ID NO:2, or (2) any DNA containing a partial base sequence of the DNA having a base sequence hybridizable to the base sequence represented by SEQ ID NO:2 under highly stringent conditions and encoding a protein which has the activities (e.g., a ligand-binding activity, a signal transduction activity, etc.) substantially equivalent to those of the protein peptide of the present invention.

Specific examples of the DNA that is hybridizable to the base sequence represented by SEQ ID NO:2 include DNA containing a base sequence having at least about 70% homology, preferably at least about 80% homology, more preferably at least about 90% homology and most preferably at least about 95% homology, most preferably at least about 98% homology, to the base sequence represented by SEQ ID NO:2.

For cloning of the DNA that completely encodes the protein of the present invention or its partial peptide (hereinafter sometimes collectively referred to as the protein of the present invention), the DNA may be either amplified by PCR using synthetic DNA primers containing a part of the base sequence of the protein of the present invention, or the DNA inserted into an

appropriate vector can be selected by hybridization with a labeled DNA fragment or synthetic DNA that encodes a part or entire region of the protein of the present invention. The hybridization can be carried out,
 5 for example, according to the method described in Molecular Cloning, 2nd, J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989. The hybridization may also be performed using commercially available library in accordance with the protocol described in the attached
 10 instructions.

Conversion of the base sequence of the DNA can be effected by publicly known methods such as the Gapped duplex method or the Kunkel method or its modification by using a publicly known kit available as MutanTM-G or
 15 MutanTM-K (both manufactured by Takara Shuzo Co., Ltd.).

The cloned DNA encoding the protein can be used as it is, depending upon purpose or, if desired, after digestion with a restriction enzyme or after addition of a linker thereto. The DNA may contain ATG as a
 20 translation initiation codon at the 5' end thereof and may further contain TAA, TGA or TAG as a translation termination codon at the 3' end thereof. These translation initiation and termination codons may also be added by using an appropriate synthetic DNA adapter.

25 The expression vector for the protein of the present invention can be manufactured, for example, by (a) excising the desired DNA fragment from the DNA encoding the protein of the present invention, and then (b) ligating the DNA fragment with an appropriate
 30 expression vector downstream a promoter in the vector.

Examples of the vector include plasmids derived from E. coli (e.g., pBR322, pBR325, pUC12, pUC13), plasmids derived from Bacillus subtilis (e.g., pUB110, pTP5, pC194), plasmids derived from yeast (e.g., pSH19,
 35 pSH15), bacteriophages such as λ phage, etc., animal

viruses such as retrovirus, vaccinia virus, baculovirus, etc. as well as pA1-11, pXT1, pRc/CMV, pRc/RSV, pCDNAI/Neo, etc.

5 The promoter used in the present invention may be any promoter if it matches well with a host to be used for gene expression. In the case of using animal cells as the host, examples of the promoter include SR α promoter, SV40 promoter, HIV-LTR promoter, CMV promoter, HSV-TK promoter, etc.

10 Among them, CMV promoter or SR α promoter is preferably used. Where the host is bacteria of the genus Escherichia, preferred examples of the promoter include trp promoter, lac promoter, recA promoter, λ P_L promoter, lpp promoter, etc. In the case of using
15 bacteria of the genus Bacillus as the host, preferred example of the promoter are SPO1 promoter, SPO2 promoter and penP promoter. When yeast is used as the host, preferred examples of the promoter are PHO5 promoter, PGK promoter, GAP promoter and ADH promoter.
20 When insect cells are used as the host, preferred examples of the promoter include polyhedrin promoter and P10 promoter.

 In addition to the foregoing examples, the expression vector may further optionally contain an
25 enhancer, a splicing signal, a poly A addition signal, a selection marker, SV40 replication origin (hereinafter sometimes abbreviated as SV40ori) etc. Examples of the selection marker include dihydrofolate reductase (hereinafter sometimes abbreviated as dhfr)
30 gene [methotrexate (MTX) resistance], ampicillin resistant gene (hereinafter sometimes abbreviated as Amp^r), neomycin resistant gene (hereinafter sometimes abbreviated as Neo^r, G418 resistance), etc. In particular, when dhfr gene is used as the selection

marker in CHO (dhfr⁻) cells, selection can also be made on thymidine free media.

If necessary and desired, a signal sequence that matches with a host is added to the N-terminus of the protein of the present invention. Examples of the signal sequence that can be used are Pho A signal sequence, OmpA signal sequence, etc. in the case of using bacteria of the genus *Escherichia* as the host; α -amylase signal sequence, subtilisin signal sequence, etc. in the case of using bacteria of the genus *Bacillus* as the host; MF α signal sequence, SUC2 signal sequence, etc. in the case of using yeast as the host; and insulin signal sequence, α -interferon signal sequence, antibody molecule signal sequence, etc. in the case of using animal cells as the host, respectively.

Using the vector containing the DNA encoding the protein of the present invention thus constructed, transformants can be manufactured.

Examples of the host, which may be employed, are bacteria belonging to the genus *Escherichia*, bacteria belonging to the genus *Bacillus*, yeast, insect cells, insects and animal cells, etc.

Specific examples of the bacteria belonging to the genus *Escherichia* include *Escherichia coli* K12 DH1 (Proc. Natl. Acad. Sci. U.S.A., 60, 160 (1968)), JM103 (Nucleic Acids Research, 9, 309 (1981)), JA221 (Journal of Molecular Biology, 120, 517 (1978)), HB101 (Journal of Molecular Biology, 41, 459 (1969)), C600 (Genetics, 39, 440 (1954)), etc.

Examples of the bacteria belonging to the genus *Bacillus* include *Bacillus subtilis* MI114 (Gene, 24, 255 (1983)), 207-21 (Journal of Biochemistry, 95, 87 (1984)), etc.

Examples of yeast include *Saccharomyces cerevisiae* AH22, AH22R⁻, NA87-11A, DKD-5D, 20B-12, *Schizosaccharomyces pombe* NCYC1913, NCYC2036, *Pichia pastoris*, etc.

5 Examples of insect cells include, for the virus AcNPV, *Spodoptera frugiperda* cells (Sf cells), MG1 cells derived from mid-intestine of *Trichoplusia ni*, High FiveTM cells derived from egg of *Trichoplusia ni*, cells derived from *Mamestra brassicae*, cells derived
10 from *Estigmena acrea*, etc.; and for the virus BmNPV, *Bombyx mori* N cells (BmN cells), etc. are used. Examples of the Sf cell which can be used are Sf9 cells (ATCC CRL1711) and Sf21 cells (both cells are described in Vaughn, J. L. et al., *In Vivo*, 13, 213-217 (1977)).

15 As the insect, for example, a larva of *Bombyx mori* can be used (Maeda, et al., *Nature*, 315, 592 (1985)).

 Examples of animal cells include monkey cells COS-7, Vero, Chinese hamster cells CHO (hereinafter referred to as CHO cells), dhfr gene deficient Chinese
20 hamster cells CHO (hereinafter simply referred to as CHO(dhfr⁻) cell), mouse L cells, mouse AtT-20, mouse myeloma cells, rat GH3, human FL cells, etc.

 Bacteria belonging to the genus *Escherichia* can be transformed, for example, by the method described in
25 Proc. Natl. Acad. Sci. U.S.A., 69, 2110 (1972) or Gene, 17, 107 (1982). Bacteria belonging to the genus *Bacillus* can be transformed, for example, by the method described in *Molecular & General Genetics*, 168, 111 (1979).

30 Yeast can be transformed, for example, by the method described in *Methods in Enzymology*, 194, 182-187 (1991), Proc. Natl. Acad. Sci. U.S.A., 75, 1929 (1978), etc.

Insect cells or insects can be transformed, for example, according to the method described in Bio/Technology, 6, 47-55(1988), etc.

Animal cells can be transformed, for example,
5 according to the method described in *Saibo Kogaku* (Cell Engineering), extra issue 8, *Shin Saibo Kogaku Jikken Protocol* (New Cell Engineering Experimental Protocol), 263-267 (1995), published by Shujunsha, or *Virology*, 52, 456 (1973).

10 Thus, the transformant transformed with the expression vector containing the DNA encoding the G protein-coupled receptor protein can be obtained.

Where the host is bacteria belonging to the genus *Escherichia* or the genus *Bacillus*, the transformant can
15 be appropriately incubated in a liquid medium which contains materials required for growth of the transformant such as carbon sources, nitrogen sources, inorganic materials, and so on. Examples of the carbon sources include glucose, dextrin, soluble starch,
20 sucrose, etc. Examples of the nitrogen sources include inorganic or organic materials such as ammonium salts, nitrate salts, corn steep liquor, peptone, casein, meat extract, soybean cake, potato extract, etc. Examples of the inorganic materials are calcium chloride, sodium
25 dihydrogenphosphate, magnesium chloride, etc. In addition, yeast, vitamins, growth promoting factors etc. may also be added to the medium. Preferably, pH of the medium is adjusted to about 5 to about 8.

A preferred example of the medium for incubation
30 of the bacteria belonging to the genus *Escherichia* is M9 medium supplemented with glucose and Casamino acids (Miller, *Journal of Experiments in Molecular Genetics*, 431-433, Cold Spring Harbor Laboratory, New York, 1972). If necessary and desired, a chemical such as 3β -

indolylacrylic acid can be added to the medium thereby to activate the promoter efficiently.

Where the bacteria belonging to the genus *Escherichia* are used as the host, the transformant is usually cultivated at about 15°C to about 43°C for about 3 hours to about 24 hours. If necessary and desired, the culture may be aerated or agitated.

Where the bacteria belonging to the genus *Bacillus* are used as the host, the transformant is cultivated generally at about 30°C to about 40°C for about 6 hours to about 24 hours. If necessary and desired, the culture can be aerated or agitated.

Where yeast is used as the host, the transformant is cultivated, for example, in Burkholder's minimal medium (Bostian, K. L. et al., Proc. Natl. Acad. Sci. U.S.A., 77, 4505 (1980)) or in SD medium supplemented with 0.5% Casamino acids (Bitter, G. A. et al., Proc. Natl. Acad. Sci. U.S.A., 81, 5330 (1984)). Preferably, pH of the medium is adjusted to about 5 to about 8. In general, the transformant is cultivated at about 20°C to about 35°C for about 24 hours to about 72 hours. If necessary and desired, the culture can be aerated or agitated.

Where insect cells or insects are used as the host, the transformant is cultivated in, for example, Grace's Insect Medium (Grace, T. C. C., Nature, 195, 788 (1962)) to which an appropriate additive such as immobilized 10% bovine serum is added. Preferably, pH of the medium is adjusted to about 6.2 to about 6.4. Normally, the transformant is cultivated at about 27°C for about 3 days to about 5 days and, if necessary and desired, the culture can be aerated or agitated.

Where animal cells are employed as the host, the transformant is cultivated in, for example, MEM medium containing about 5% to about 20% fetal bovine serum

(Science, 122, 501 (1952)), DMEM medium (Virology, 8, 396 (1959)), RPMI 1640 medium (The Journal of the American Medical Association, 199, 519 (1967)), 199 medium (Proceeding of the Society for the Biological
5 Medicine, 73, 1 (1950)), etc. Preferably, pH of the medium is adjusted to about 6 to about 8. The transformant is usually cultivated at about 30°C to about 40°C for about 15 hours to about 60 hours and, if necessary and desired, the culture can be aerated or
10 agitated.

As described above, the G protein-coupled receptor protein of the present invention can be produced in the cell membrane of the transformant, etc.

The protein of the present invention can be
15 separated and purified from the culture described above by the following procedures.

When the protein of the present invention is extracted from the culture or cells, after cultivation the transformants or cells are collected by a publicly
20 known method and suspended in an appropriate buffer. The transformants or cells are then disrupted by publicly known methods such as ultrasonication, a treatment with lysozyme and/or freeze-thaw cycling, followed by centrifugation, filtration, etc. Thus, the
25 crude extract of the protein of the present invention can be obtained. The buffer used for the procedures may contain a protein modifier such as urea or guanidine hydrochloride, or a surfactant such as Triton X-100TM, etc. When the protein is secreted in the culture, after
30 completion of the cultivation the supernatant can be separated from the transformants or cells to collect the supernatant by a publicly known method.

The protein contained in the supernatant or the extract thus obtained can be purified by appropriately
35 combining the publicly known methods for separation and

purification. Such publicly known methods for separation and purification include a method utilizing difference in solubility such as salting out, solvent precipitation, etc.; a method utilizing mainly
5 difference in molecular weight such as dialysis, ultrafiltration, gel filtration, SDS-polyacrylamide gel electrophoresis, etc.; a method utilizing difference in electric charge such as ion exchange chromatography, etc.; a method utilizing difference in specific
10 affinity such as affinity chromatography, etc.; a method utilizing difference in hydrophobicity such as reverse phase high performance liquid chromatography, etc.; a method utilizing difference in isoelectric point such as isoelectrofocusing electrophoresis; and
15 the like.

When the protein thus obtained is in a free form, it can be converted into the salt by publicly known methods or modifications thereof. On the other hand, when the protein is obtained in the form of a salt, it
20 can be converted into the free form or in the form of a different salt by publicly known methods or modifications thereof.

The protein produced by the recombinant can be treated, prior to or after the purification, with an
25 appropriate protein modifying enzyme so that the protein can be appropriately modified to partially remove a polypeptide. Examples of the protein-modifying enzyme include trypsin, chymotrypsin, arginyl endopeptidase, protein kinase, glycosidase or the like.

30 The activity of the thus produced protein of the present invention or salts thereof can be determined by a test binding to a labeled ligand, by an enzyme immunoassay using a specific antibody, or the like.

Antibodies to the protein of the present invention,
35 its partial peptides, or salts thereof may be any of

polyclonal antibodies and monoclonal antibodies, as long as they are capable of recognizing the protein of the present invention, its partial peptides, or salts thereof.

5 The antibodies to the protein of the present invention, its partial peptides, or salts thereof (hereinafter sometimes merely referred to as the protein of the present invention) may be manufactured by publicly known methods for manufacturing antibodies
10 or antisera, using as antigens the protein of the present invention.

[Preparation of monoclonal antibody]

(a) Preparation of monoclonal antibody-producing cells

15 The polypeptide or protein of the present invention is administered to warm-blooded animals either solely or together with carriers or diluents to the site where the production of antibody is possible by the administration. In order to potentiate the
20 antibody productivity upon the administration, complete Freund's adjuvants or incomplete Freund's adjuvants may be administered. The administration is usually carried out once every two to six weeks and two to ten times in total. Examples of the applicable warm-blooded animals
25 are monkeys, rabbits, dogs, guinea pigs, mice, rats, sheep and goats, with the use of mice and rats being preferred.

 In the preparation of monoclonal antibody-producing cells, a warm-blooded animal, e.g., mice,
30 immunized with an antigen wherein the antibody titer is noted is selected, then spleen or lymph node is collected after two to five days from the final immunization and antibody-producing cells contained therein are fused with myeloma cells from homozygous or
35 heterozygous animal to give monoclonal antibody-producing

hybridomas. Measurement of the antibody titer in antisera may be carried out, for example, by reacting a labeled polypeptide, which will be described later, with the antiserum followed by assaying the binding activity of the labeling agent bound to the antibody. The fusion may be carried out, for example, by the known method by Koehler and Milstein (Nature, 256, 495, 1975). Examples of the fusion accelerator are polyethylene glycol (PEG), Sendai virus, etc., of which PEG is preferably employed.

Examples of the myeloma cells are those collected from warm-blooded animals such as NS-1, P3U1, SP2/0, AP-1, etc. In particular, P3U1 is preferably employed. A preferred ratio of the count of the antibody-producing cells used (spleen cells) to the count of myeloma cells is within a range of approximately 1:1 to 20:1. When PEG (preferably, PEG 1000 to PEG 6000) is added in a concentration of approximately 10 to 80% followed by incubating at 20 to 40°C, preferably at 30 to 37°C for 1 to 10 minutes, an efficient cell fusion can be carried out.

Various methods can be used for screening of a monoclonal antibody-producing hybridoma. Examples of such methods include a method which comprises adding the supernatant of hybridoma to a solid phase (e.g., microplate) adsorbed with the polypeptide (protein) as an antigen directly or together with a carrier, adding an anti-immunoglobulin antibody (where mouse cells are used for the cell fusion, anti-mouse immunoglobulin antibody is used) labeled with a radioactive substance or an enzyme or Protein A and detecting the monoclonal antibody bound to the solid phase, and a method which comprises adding the supernatant of hybridoma to a solid phase adsorbed with an anti-immunoglobulin antibody or Protein A, adding the polypeptide labeled

with a radioactive substance or an enzyme and detecting the monoclonal antibody bound to the solid phase.

The monoclonal antibody can be selected according to publicly known methods or their modifications. In
5 general, the selection can be effected in a medium for animal cells supplemented with HAT (hypoxanthine, aminopterin and thymidine). Any selection and growth medium can be employed as far as the hybridoma can grow there. For example, RPMI 1640 medium containing 1% to
10 20%, preferably 10% to 20% fetal bovine serum, GIT medium (Wako Pure Chemical Industries, Ltd.) containing 1% to 10% fetal bovine serum, a serum free medium for cultivation of a hybridoma (SFM-101, Nissui Seiyaku Co., Ltd.) and the like can be used for the selection and
15 growth medium. The cultivation is carried out generally at 20°C to 40°C, preferably at 37°C, for about 5 days to about 3 weeks, preferably 1 to 2 weeks, normally in 5% CO₂. The antibody titer of the culture supernatant of a hybridoma can be determined as in the assay for
20 the antibody titer in antisera described above.

(b) Purification of monoclonal antibody

Separation and purification of a monoclonal antibody can be carried out by publicly known methods, such as separation and purification of immunoglobulins
25 (for example, salting-out, alcohol precipitation, isoelectric point precipitation, electrophoresis, adsorption and desorption with ion exchangers (e.g., DEAE), ultracentrifugation, gel filtration, or a specific purification method which comprises collecting
30 only an antibody with an activated adsorbent such as an antigen-binding solid phase, Protein A or Protein G and dissociating the binding to obtain the antibody.

[Preparation of polyclonal antibody]

The polyclonal antibody of the present invention can be manufactured by publicly known methods or modifications thereof. For example, a warm-blooded animal is immunized with an immunogen (protein antigen) per se, or a complex of immunogen and a carrier protein is formed and a warm-blooded animal is immunized with the complex in a manner similar to the method described above for the manufacture of monoclonal antibodies. The product containing the antibody to the polypeptide of the present invention is collected from the immunized animal followed by separation and purification of the antibody.

In the complex of immunogen and carrier protein used to immunize a warm-blooded animal, the type of carrier protein and the mixing ratio of carrier to hapten may be any type and in any ratio, as long as the antibody is efficiently produced to the hapten immunized by crosslinking to the carrier. For example, bovine serum albumin, bovine thyroglobulin or hemocyanin is coupled to hapten in a carrier-to-hapten weight ratio of approximately 0.1 to 20, preferably about 1 to about 5.

A variety of condensation agents can be used for the coupling of carrier to hapten. Glutaraldehyde, carbodiimide, maleimide activated ester and activated ester reagents containing thiol group or dithiopyridyl group are used for the coupling.

The condensation product is administered to warm-blooded animals either solely or together with carriers or diluents to the site that can produce the antibody by the administration. In order to potentiate the antibody productivity upon the administration, complete Freund's adjuvant or incomplete Freund's adjuvant may be administered. The administration is usually made once every 2 to 6 weeks and 3 to 10 times in total.

The polyclonal antibody can be collected from the blood, ascites, etc., preferably from the blood of warm-blooded animal immunized by the method described above.

5 The polyclonal antibody titer in antiserum can be assayed by the same procedure as that for the determination of serum antibody titer described above. The separation and purification of the polyclonal antibody can be carried out, following the method for
10 the separation and purification of immunoglobulins performed as in the separation and purification of monoclonal antibodies described hereinabove.

 The protein of the present invention, its partial peptides, or salts thereof and the DNA encoding the
15 same can be used for; ① a determination method of ligands to the protein of the present invention; ② preparation of antibodies and antisera; ③ construction of recombinant protein expression systems; ④ development of the receptor binding assay systems using
20 the expression systems and screening of pharmaceutical candidate compounds; ⑤ effecting drug design based on comparison with structurally similar ligand receptors; ⑥ reagents for preparation of probes and PCR primers for gene diagnosis; ⑦ production of transgenic
25 animals; and ⑧ pharmaceutical drugs for the gene prophylaxis and gene therapy.

 In particular, by the use of the receptor binding assay system using the expression system of the recombinant G protein-coupled receptor protein of the
30 present invention, compounds (e.g., agonists, antagonists, etc.) that alter the binding property of human- or mammal-specific ligands for the G protein-coupled receptor protein can be screened, and the agonists or antagonists can be used as prophylactic and
35 therapeutic agents for various diseases.

Hereinafter, the protein of the present invention, its partial peptides, or salts thereof (hereinafter sometimes referred to as the protein of the present invention), the DNA encoding the protein of the present invention or its partial peptides (hereinafter sometimes referred to as the DNA of the present invention) and the antibodies to the protein of the present invention (hereinafter sometimes referred to as the antibodies of the present invention) are specifically described for the use or applications.

(1) Determination of a ligand (agonist) to the protein of the present invention

The protein of the present invention or its salts, or the partial peptide or its salts of the present invention are useful as reagents for searching and determining ligands (agonists) to the protein of the present invention or its salts.

That is, the present invention provides a method for determining a ligand to the protein of the present invention, which comprises bringing the protein of the present invention or its salts, or the partial peptide of the present invention or its salts, in contact with a test compound.

Examples of the test compound include publicly known ligands (e.g., angiotensin, bombesin, canavanoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purines, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedulin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal and related polypeptide), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene-related peptide), leukotrienes, pancreastatin, prostaglandins, thromboxane, adenosine, adrenaline, α and β -chemokines (e.g., IL-8, GRO α , GRO β ,

GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP-1 α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptide, galanin, etc.) as well as other substances, for example, tissue extracts and cell culture supernatants from human and mammals (e.g., mice, rats, swine, bovine, sheep, monkeys, etc.). For example, the tissue extract or cell culture supernatant is added to the protein of the present invention and fractionated while assaying the cell stimulating activities, etc. to finally give a single ligand.

In more detail, the method for determining ligands of the present invention comprises determining compounds (e.g., peptides, proteins, non-peptide compounds, synthetic compounds, fermentation products, etc.) or salts thereof that bind to the protein of the present invention to provide cell stimulating activities (e.g., the activities that accelerate or suppress arachidonic acid release, acetylcholine release, intracellular Ca²⁺ release, intracellular cAMP production, intracellular cGMP production, inositol phosphate production, change in cell membrane potential, phosphorylation of intracellular proteins, activation of c-fos, pH reduction, etc.), using the protein of the present invention, its partial peptides or salts thereof, or by the receptor binding assay using the constructed recombinant protein expression system.

The method for determining ligands of the present invention is characterized, for example, by measurement of the amount of the test compound bound to the protein or the partial peptide, or by assaying the cell-stimulating activities, etc., when the test compound is brought in contact with the protein of the present invention or its partial peptides.

More specifically, the present invention provides the following:

- 5 (1) a method for determining a ligand to the protein of the present invention or its salt, which comprises bringing a labeled test compound in contact with the protein of the present invention or its salt or the partial peptide of the present invention or its salt and measuring the amount of the labeled test compound bound to the protein or its salt or to the partial
10 peptide or its salt;
- (2) a method for determining ligands to the protein of the present invention or its salt, which comprises bringing a labeled test compound in contact with cells or cell membrane fraction containing the protein of the
15 present invention, and measuring the amount of the labeled test compound bound to the cells or the membrane fraction;
- (3) a method for determining ligands to the protein of the present invention, which comprises culturing a
20 transformant containing the DNA encoding the protein of the present invention, bringing a labeled test compound in contact with the receptor protein expressed on the cell membrane by said culturing, and measuring the amount of the labeled test compound bound to the
25 protein or its salt;
- (4) a method for determining ligands to the protein of the present invention or its salt, which comprises bringing a test compound in contact with cells containing the protein of the present invention and
30 measuring the protein-mediated cell stimulating activities (e.g., the activities that promote or suppress arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP production, intracellular cGMP production, inositol
35 phosphate production, change in cell membrane potential,

phosphorylation of intracellular proteins, activation of c-fos, pH reduction, etc.); and,

(5) a method for determining ligands to the protein of the present invention or its salt, which comprises
5 culturing a transformant containing DNA encoding the protein of the present invention, bringing a labeled test compound in contact with the protein expressed on the cell membrane by said culturing, and measuring the protein-mediated cell stimulating activities (e.g., the
10 activities that promote or suppress arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP production, intracellular cGMP production, inositol phosphate production, change in cell membrane potential, phosphorylation of
15 intracellular proteins, activation of c-fos, pH reduction, etc.).

It is particularly preferred to perform the tests (1) to (3) described above, thereby to confirm that the test compound can bind to the protein of the present
20 invention, followed by the tests (4) and (5) described above.

Any protein exemplified to be usable as the receptor protein for determining ligands, so long as it contains the protein of the present invention or the
25 partial peptide of the present invention. However, the protein that is abundantly expressed using animal cells is appropriate.

The protein of the present invention can be manufactured by the method for expression described
30 above, preferably by expressing DNA encoding the protein in mammalian or insect cells. As DNA fragments encoding the desired portion of the protein, complementary DNA is generally used but not necessarily limited thereto. For example, gene fragments or
35 synthetic DNA may also be used. For introducing a DNA

fragment encoding the protein of the present invention into host animal cells and efficiently expressing the same, it is preferred to insert the DNA fragment downstream a polyhedrin promoter of nuclear
5 polyhedrosis virus (NPV), which is a baculovirus having insect hosts, an SV40-derived promoter, a retrovirus promoter, a metallothionein promoter, a human heat shock promoter, a cytomegalovirus promoter, an SR α promoter or the like. The amount and quality of the
10 receptor expressed can be determined by a publicly known method. For example, this determination can be made by the method described in the literature (Nambi, P., et al., J. Biol. Chem., 267, 19555-19559 (1992)).

Accordingly, the subject containing the protein of
15 the present invention, its partial peptides or salts thereof in the method for determining the ligand according to the present invention may be the protein, its partial peptides or salts thereof purified by publicly known methods, cells containing the protein,
20 or membrane fractions of such cells.

Where cells containing the protein of the present invention are used in the method of the present invention for determination of ligands, the cells may be fixed using glutaraldehyde, formalin, etc. The
25 fixation can be made by a publicly known method.

The cells containing the protein of the present invention are host cells that have expressed the protein of the present invention, which host cells include Escherichia coli, Bacillus subtilis, yeast,
30 insect cells, animal cells, and the like.

The cell membrane fraction refers to a fraction abundant in cell membrane obtained by cell disruption and subsequent fractionation by a publicly known method. Useful cell disruption methods include cell squashing
35 using a Potter-Elvehjem homogenizer, disruption using a

Waring blender or Polytron (manufactured by Kinematica Inc.), disruption by ultrasonication, and disruption by cell spraying through thin nozzles under an increased pressure using a French press or the like. Cell
5 membrane fractionation is effected mainly by fractionation using a centrifugal force, such as centrifugation for fractionation and density gradient centrifugation. For example, cell disruption fluid is centrifuged at a low speed (500 rpm to 3,000 rpm) for a
10 short period of time (normally about 1 to about 10 minutes), the resulting supernatant is then centrifuged at a higher speed (15,000 rpm to 30,000 rpm) normally for 30 minutes to 2 hours. The precipitate thus obtained is used as the membrane fraction. The
15 membrane fraction is rich in the protein expressed and membrane components such as cell-derived phospholipids and membrane proteins.

The amount of the protein in the cells containing the protein and in the membrane fraction is preferably
20 10^3 to 10^8 molecules per cell, more preferably 10^5 to 10^7 molecules per cell. As the amount of expression increases, the ligand binding activity per unit of membrane fraction (specific activity) increases so that not only the highly sensitive screening system can be
25 constructed but also large quantities of samples can be assayed with the same lot.

To perform the methods (1) through (3) supra for determination of a ligand to the protein of the present invention or its salt, an appropriate protein fraction
30 and a labeled test compound are required.

The protein fraction is preferably a fraction of naturally occurring receptor protein or a recombinant receptor fraction having an activity equivalent to that of the natural protein. Herein, the term "equivalent
35 activity" is intended to mean a ligand binding activity,

a signal transduction activity or the like that is equivalent to that possessed by naturally occurring receptor proteins.

Preferred examples of labeled test compounds
5 include angiotensin, bombesin, canavaninoid, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purines, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP
10 (vasoactive intestinal polypeptide), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene-related peptide), leukotrienes, pancreastatin, prostaglandins, thromboxane, adenosine, adrenaline, α and β -chemokines (e.g., IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309,
15 MIP1 α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamin, neurotensin, TRH, pancreatic polypeptide, galanin, etc.), which are labeled with [^3H], [^{125}I], [^{14}C], [^{35}S], etc.

20 More specifically, the ligand to the protein of the present invention or its salt is determined by the following procedures. First, a standard receptor preparation is prepared by suspending cells containing the protein of the present invention or the membrane
25 fraction thereof in a buffer appropriate for use in the determination method. Any buffer can be used so long as it does not inhibit the ligand-receptor binding, such buffers including a phosphate buffer or a Tris-HCl buffer having pH of 4 to 10 (preferably pH of 6 to 8).
30 For the purpose of minimizing non-specific binding, a surfactant such as CHAPS, Tween-80TM (manufactured by Kao-Atlas Inc.), digitonin or deoxycholate, and various proteins such as bovine serum albumin or gelatin, may optionally be added to the buffer. Further for the
35 purpose of suppressing the degradation of the receptors

or ligands by proteases, a protease inhibitor such as PMSF, leupeptin, E-64 (manufactured by Peptide Institute, Inc.) and pepstatin may also be added. A given amount (5,000 to 500,000 cpm) of the test
5 compound labeled with [³H], [¹²⁵I], [¹⁴C], [³⁵S] or the like is added to 0.01 ml to 10 ml of the receptor solution. To determine the amount of non-specific binding (NSB), a reaction tube containing an unlabeled test compound in a large excess is also prepared. The
10 reaction is carried out at approximately 0 to 50°C, preferably about 4 to 37°C for about 20 minutes to about 24 hours, preferably about 30 minutes to about 3 hours. After completion of the reaction, the reaction mixture is filtrated through glass fiber filter paper,
15 etc. and washed with an appropriate volume of the same buffer. The residual radioactivity on the glass fiber filter paper is then measured by means of a liquid scintillation counter or γ-counter. A test compound exceeding 0 cpm in count obtained by subtracting
20 nonspecific binding (NSB) from the total binding (B) (B minus NSB) may be selected as a ligand (agonist) to the protein of the present invention or its salt.

The method (4) or (5) above for determination of a ligand to the protein of the present invention or its
25 salt can be performed as follows. The protein-mediated cell-stimulating activities (e.g., the activities that promote or suppress arachidonic acid release, acetylcholine release, intracellular Ca²⁺ release, intracellular cAMP production, intracellular cGMP
30 production, inositol phosphate production, change in cell membrane potential, phosphorylation of intracellular proteins, activation of c-fos, pH reduction, etc.) may be determined by a publicly known method, or using an assay kit commercially available.
35 Specifically, cells containing the protein are first

cultured on a multi-well plate, etc. Prior to the ligand determination, the medium is replaced with fresh medium or with an appropriate non-cytotoxic buffer, followed by incubation for a given period of time in the presence of a test compound, etc. Subsequently, the cells are extracted or the supernatant is recovered and the resulting product is quantified by appropriate procedures. Where it is difficult to detect the production of the index substance (e.g., arachidonic acid) for the cell-stimulating activity due to a degrading enzyme contained in the cells, an inhibitor against such a degrading enzyme may be added prior to the assay. For detecting activities such as the cAMP production suppression activity, the baseline production in the cells is increased by forskolin or the like and the suppressing effect on the increased baseline production may then be detected.

The kit of the present invention for determination of the ligand that binds to the protein of the present invention or its salt comprises the protein of the present invention or its salt, the partial peptide of the present invention or its salt, cells containing the protein of the present invention, or the membrane fraction of the cells containing the protein of the present invention.

Examples of the ligand determination kit of the present invention are given below.

1. Reagents for determining ligands
- (1) Buffers for assay and washing

Hanks' Balanced Salt Solution (manufactured by Gibco Co.) supplemented with 0.05% bovine serum albumin (Sigma Co.).

The solution is sterilized by filtration through a 0.45 μm filter and stored at 4°C. Alternatively, the solution may be prepared at use.

(2) Standard G protein-coupled receptor protein

5 CHO cells on which the protein of the present invention has been expressed are passaged in a 12-well plate in a density of 5×10^5 cells/well followed by culturing at 37°C under 5% CO_2 and 95% air for 2 days.

(3) Labeled test compounds

10 Compounds labeled with [^3H], [^{125}I], [^{14}C], [^{35}S], etc., which are commercially available labels, or compounds labeled by appropriate methods.

An aqueous solution of the compound is stored at 4°C or -20°C. The solution is diluted to 1 μM with an assay buffer at use. A sparingly water-soluble test compound is dissolved in dimethylformamide, DMSO, methanol, etc.

(4) Non-labeled compounds

20 A non-labeled form of the same compound as the labeled compound is prepared in a concentration 100 to 1,000-fold higher than that of the labeled compound.

2. Method for assay

25 (1) CHO cells expressing the protein of the present invention are cultured in a 12-well culture plate. After washing twice with 1 ml of an assay buffer, 490 μl of the assay buffer is added to each well.

(2) After 5 μl of the labeled test compound is added, the resulting mixture is incubated at room temperature for an hour. To determine the non-specific binding, 5 μl of the non-labeled compound is added to the system.

35 (3) The reaction mixture is removed and the wells are washed 3 times with 1 ml of washing buffer. The labeled test compound bound to the cells is dissolved

in 0.2N NaOH-1% SDS and then mixed with 4 ml of liquid scintillator A (manufactured by Wako Pure Chemical Industries, Ltd.).

(4) The radioactivity is measured using a liquid scintillation counter (manufactured by Beckman Co.).

The ligands that bind to the protein of the present invention or its salt include substances specifically present in the brain, pituitary gland and pancreas. Examples of such ligands are angiotensin, bombesin, canavanine, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioids, purines, vasopressin, oxytocin, PACAP, secretin, glucagon, calcitonin, adrenomedullin, somatostatin, GHRH, CRF, ACTH, GRP, PTH, VIP (vasoactive intestinal peptide), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene-related peptide), leukotrienes, pancreastatin, prostaglandins, thromboxane, adenosine, adrenaline, α and β -chemokines (e.g., IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic polypeptide, galanin, etc.

(2) Prophylactic and/or therapeutic agents for diseases associated with lack of the G protein-coupled receptor protein of the present invention

When a compound is clarified to be a ligand of the protein of the present invention by the methods described in (1), ① the protein of the present invention, or ② the DNA encoding the protein can be used, depending on the activities possessed by the ligand, as a prophylactic and/or therapeutic agent for diseases associated with dysfunction of the protein of the present invention.

For example, when the physiological activity of the ligand cannot be expected in a patient (deficiency of the protein) due to a decrease in the protein of the present invention, the activity of the ligand can be exhibited by: ① administering the protein of the present invention to the patient thereby to supplement the amount of the protein; or ② by increasing the amount of the protein in the patient through: i) administration of the DNA encoding the protein of the present invention to express the same in the patient; or ii) insertion and expression of the DNA encoding the protein of the present invention in the objective cells to transplant the cells to the patient, whereby the activity of the ligand can be sufficiently exhibited. That is, the DNA encoding the protein of the present invention is useful as a safe and low toxic prophylactic and/or therapeutic agent for diseases associated with dysfunction of the protein of the present invention.

The protein of the present invention and the DNA encoding the protein of the present invention are useful for the prevention and/or treatment of central dysfunction (e.g., Alzheimer's disease, senile dementia, suppression of eating (anorexia), epilepsy, etc.), hormone diseases (e.g., weak pains, atonic bleeding, before and after expulsion, subinvolution of uterus, cesarean section, induced abortion, galactostasis, etc.), liver/gallbladder/pancreas/endocrine-associated diseases (e.g., diabetes mellitus, suppression of eating, etc.), inflammatory diseases (e.g., allergy, asthma, rheumatoid, etc.), circulatory diseases (e.g., hypertension, cardiac hypertrophy, angina pectoris, arteriosclerosis, etc.).

When the protein of the present invention is used as the prophylactic/therapeutic agents supra, the

protein can be prepared into a pharmaceutical composition in a conventional manner.

On the other hand, where the DNA encoding the protein of the present invention (hereinafter sometimes referred to as the DNA of the present invention) is used as the prophylactic/therapeutic agents described above, the DNA itself is administered; alternatively, the DNA is inserted into an appropriate vector such as retrovirus vector, adenovirus vector, adenovirus-associated virus vector, etc. and then administered in a conventional manner. The DNA of the present invention may also be administered as naked DNA, or with adjuvants to assist its uptake by gene gun or through a catheter such as a catheter with a hydrogel.

For example, ① the protein of the present invention or ② the DNA encoding the protein can be used orally, for example, in the form of tablets which may be sugar coated if necessary and desired, capsules, elixirs, microcapsules etc., or parenterally in the form of injectable preparations such as a sterile solution and a suspension in water or with other pharmaceutically acceptable liquid. These preparations can be manufactured by mixing ① the protein of the present invention or ② the DNA encoding the protein with a physiologically acceptable known carrier, a flavoring agent, an excipient, a vehicle, an antiseptic agent, a stabilizer, a binder, etc. in a unit dosage form required in a generally accepted manner that is applied to making pharmaceutical preparations. The effective component in the preparation is controlled in such a dose that an appropriate dose is obtained within the specified range given.

Additives miscible with tablets, capsules, etc. include a binder such as gelatin, corn starch, tragacanth and gum arabic, an excipient such as

crystalline cellulose, a swelling agent such as corn starch, gelatin and alginic acid, a lubricant such as magnesium stearate, a sweetening agent such as sucrose, lactose and saccharin, and a flavoring agent such as peppermint, akamono oil and cherry. When the unit dosage is in the form of capsules, liquid carriers such as oils and fats may further be used together with the additives described above. A sterile composition for injection may be formulated by conventional procedures used to make pharmaceutical compositions, e.g., by dissolving or suspending the active ingredients in a vehicle such as water for injection with a naturally occurring vegetable oil such as sesame oil and coconut oil, etc. to prepare the pharmaceutical composition. Examples of an aqueous medium for injection include physiological saline and an isotonic solution containing glucose and other auxiliary agents (e.g., D-sorbitol, D-mannitol, sodium chloride, etc.) and may be used in combination with an appropriate dissolution aid such as an alcohol (e.g., ethanol or the like), a polyalcohol (e.g., propylene glycol and polyethylene glycol), a nonionic surfactant (e.g., polysorbate 80TM and HCO-50), etc. Examples of the oily medium include sesame oil and soybean oil, which may also be used in combination with a dissolution aid such as benzyl benzoate and benzyl alcohol.

The prophylactic/therapeutic agent described above may further be formulated with a buffer (e.g., phosphate buffer, sodium acetate buffer, etc.), a soothing agent (e.g., benzalkonium chloride, procaine hydrochloride, etc.), a stabilizer (e.g., human serum albumin, polyethylene glycol, etc.), a preservative (e.g., benzyl alcohol, phenol, etc.), an antioxidant, etc. The thus-prepared liquid for injection is normally filled in an appropriate ampoule.

Since the thus obtained pharmaceutical preparation is safe and low toxic, the preparation can be administered to human or mammal (e.g., rats, rabbits, sheep, swine, bovine, cats, dogs, monkeys, etc.).

5 The dose of the protein or DNA of the present invention varies depending on subject to be administered, organs to be administered, conditions, routes for administration, etc.; in oral administration, e.g., for the adult patient with suppression of eating, 10 the dose is normally about 0.1 mg to about 100 mg, preferably about 1.0 to about 50 mg, and more preferably about 1.0 to about 20 mg per day (as 60 kg body weight). In parenteral administration, the single dose varies depending on subject to be administered, 15 target organ, conditions, routes for administration, etc., but it is advantageous, e.g., for the adult patient with suppression of eating, to administer the active ingredient intravenously in a daily dose of about 0.01 to about 30 mg, preferably about 0.1 to 20 about 20 mg, and more preferably about 0.1 to about 10 mg (as 60 kg body weight). For other animal species, the corresponding dose as converted per 60 kg body weight can be administered.

25 (3) Gene diagnostic agent

By using the DNA of the present invention as a probe, an abnormality (gene abnormality) of the DNA or mRNA encoding the protein of the present invention or its partial peptide in human or mammal (e.g., rats, 30 rabbits, sheep, swine, bovine, cats, dogs, monkeys, etc.) can be detected. Therefore, the DNA of the present invention is useful as a gene diagnostic agent for the damage against the DNA or mRNA, its mutation, or its decreased expression, or increased expression or 35 overexpression of the DNA or mRNA.

30 Using the protein of the present invention, or
using the receptor binding assay system of the
expression system constructed using the recombinant
protein, compounds (e.g., peptides, proteins, non-
peptide compounds, synthetic compounds, fermentation
35 products, etc.) or salt forms thereof that alter the

binding property between ligands and the protein of the present invention can be efficiently screened.

Such compounds include (a) compounds that have the G protein-coupled receptor-mediated cell-stimulating activities (e.g., activities that promote or suppress arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP production, intracellular cGMP production, inositol phosphate production, changes in cell membrane potential, phosphorylation of intracellular proteins, activation of c-fos, pH reduction, etc.) (so-called agonists to the protein of the present invention); (b) compounds that do not have the cell-stimulating activity (so-called antagonists to the protein of the present invention); (c) compounds that potentiate the binding affinity between ligands and the protein of the present invention; and (d) compounds that reduce the binding affinity between ligands and the protein of the present invention (it is preferred to screen the compounds described in (a) using the ligand determination methods described above).

That is, the present invention provides methods of screening compounds or their salt forms that alter the binding property between ligands and the protein, its partial peptide or salts thereof, which comprises comparing (i) the case wherein the protein of the present invention, its partial peptide or salts thereof are brought in contact with a ligand, with (ii) the case wherein the protein of the present invention, its partial peptide or salts thereof are brought in contact with a ligand and a test compound.

The screening methods of the present invention are characterized by assaying, for example, the amount of ligand bound to the protein, the cell-stimulating

activity, etc., and comparing the property between (i) and (ii).

More specifically, the present invention provides the following screening methods:

- 5 ① a method of screening a compound or its salt that alters the binding property between a ligand and the protein of the present invention, which comprises:
 measuring the amount of a labeled ligand bound to the protein, when the labeled ligand is brought in
10 contact with the protein of the present invention and when the labeled ligand and a test compound are brought in contact with the protein of the present invention, and,
 comparing the binding property between them;
- 15 ② a method of screening a compound or its salt that alters the binding property between a ligand and the protein of the present invention, which comprises:
 measuring the amount of a labeled ligand bound to cells or the membrane fraction of the cells, when the
20 labeled ligand is brought in contact with the cells or cell membrane fraction containing the protein of the present invention and when the labeled ligand and a test compound are brought in contact with the cells or cell membrane fraction containing the protein of the
25 present invention, and,
 comparing the binding property between them;
- 30 ③ a method of screening a compound or its salt that alters the binding property between a ligand and the protein of the present invention, which comprises:
 measuring the amount of a labeled ligand bound to the protein, when the labeled ligand is brought in contact with the protein expressed on the cell membrane induced by culturing a transformant containing the DNA of the present invention and when the labeled ligand
35 and a test compound are brought in contact with the

protein of the present invention expressed on the cell membrane induced by culturing a transformant containing the DNA of the present invention, and, comparing the binding property between them;

- 5 ④ a method of screening a compound or its salt that alters the binding property between a ligand and the protein of the present invention, which comprises:

 measuring the receptor-mediated cell-stimulating activity (e.g., the activity that promotes or
10 suppresses arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP production, intracellular cGMP production, inositol phosphate production, changes in cell membrane potential, phosphorylation of intracellular proteins,
15 activation of c-fos, pH reduction, etc.), when a compound (e.g., a ligand to the protein of the present invention) that activates the protein of the present invention is brought in contact with cells containing the protein of the present invention and when the
20 compound that activates the protein of the present invention and a test compound are brought in contact with cells containing the protein of the present invention, and,

- comparing the binding property between them; and,
25 ⑤ a method of screening a compound or its salt that alters the binding property between a ligand and the protein of the present invention, which comprises:

 measuring the receptor-mediated cell-stimulating activity (e.g., the activity that promotes or
30 suppresses arachidonic acid release, acetylcholine release, intracellular Ca^{2+} release, intracellular cAMP production, intracellular cGMP production, inositol phosphate production, changes in cell membrane potential, phosphorylation of intracellular proteins,
35 activation of c-fos, pH reduction, etc.), when a

compound (e.g., a ligand for the protein of the present invention) that activates the protein of the present invention is brought in contact with the protein of the present invention expressed on the cell membrane
5 induced by culturing a transformant containing the DNA of the present invention and when the compound that activates the protein of the present invention and a test compound are brought in contact with the protein of the present invention expressed on the cell membrane
10 induced by culturing a transformant containing the DNA of the present invention, and,

comparing the binding property between them.

Before the protein of the present invention was obtained, it was required for screening G protein-coupled receptor agonists or antagonists to obtain
15 candidate compounds first, using cells or tissues containing the G protein-coupled receptor protein or the cell membrane fraction from rats or other animals (primary screening), and then examine the candidate
20 compounds whether the compounds actually inhibit the binding between human G protein-coupled receptor protein and ligands (secondary screening). When cells, tissues, or the cell membrane fractions were directly used, it was practically difficult to screen agonists
25 or antagonists to the objective protein, since other receptor proteins were present together.

However, using, for example, the human-derived protein of the present invention, the primary screening becomes unnecessary, and compounds that inhibit the
30 binding between ligands and the G protein-coupled receptor protein can be efficiently screened. Furthermore, it is easy to assess whether the obtained compound is an agonist or antagonist.

Hereinafter, the screening methods of the present
35 invention are described more specifically.

First, for the protein of the present invention used for the screening methods of the present invention, any substance may be used so long as it contains the protein of the present invention described above. The
5 cell membrane fraction from mammalian organs containing the protein of the present invention is preferred. However, it is very difficult to obtain human organs. It is thus preferable to use rat-derived receptor proteins or the like, produced by large-scale
10 expression using recombinants.

To manufacture the protein of the present invention, the methods described above are used, and it is preferred to express the DNA of the present invention in mammalian and insect cells. For the DNA
15 fragment encoding the objective protein region, the complementary DNA, but not necessarily limited thereto, is employed. For example, the gene fragments and synthetic DNA may also be used. To introduce a DNA fragment encoding the protein of the present invention
20 into host animal cells and efficiently express the DNA there, it is preferred to insert the DNA fragment downstream of a polyhedrin promoter of nuclear polyhedrosis virus (NPV) belonging to baculovirus hosted by insects, SV40-derived promoter, retrovirus
25 promoter, metallothionein promoter, human heat shock promoter, cytomegalovirus promoter, or SR α promoter. The amount and quality of the expressed receptor are examined by publicly known methods, for example, the method described in the literature [Nambi, P. et al.,
30 The Journal of Biological Chemistry (J. Biol. Chem.), 267, 19555-19559, 1992].

Therefore, in the screening methods of the present invention, the material that contains the protein of the present invention may be the protein purified by

publicly known methods, cells containing the protein, or the cell membrane fraction containing the protein.

In the screening methods of the present invention, when cells containing the protein of the present
5 invention are used, the cells may be fixed with glutaraldehyde, formalin, etc. The cells can be fixed by publicly known methods.

The cells containing the protein of the present invention are host cells that express the protein. For
10 the host cells, *Escherichia coli*, *Bacillus subtilis*, yeast, insect cells, animal cells and the like are preferred.

The cell membrane fraction refers to a fraction abundant in cell membrane obtained by cell disruption
15 and subsequent fractionation by a publicly known method. Useful cell disruption methods include cell squashing using a Potter-Elvehjem homogenizer, disruption using a Waring blender or Polytron (manufactured by Kinematica Inc.), disruption by ultrasonication, and disruption by
20 cell spraying through thin nozzles under an increased pressure using a French press or the like. Cell membrane fractionation is effected mainly by fractionation using a centrifugal force, such as centrifugation for fractionation and density gradient
25 centrifugation. For example, cell disruption fluid is centrifuged at a low speed (500 rpm to 3,000 rpm) for a short period of time (normally about 1 to about 10 minutes), the resulting supernatant is then centrifuged at a higher speed (15,000 rpm to 30,000 rpm) normally
30 for 30 minutes to 2 hours. The precipitate thus obtained is used as the membrane fraction. The membrane fraction is rich in the protein expressed and membrane components such as cell-derived phospholipids and membrane proteins.

The amount of the protein in the cells containing the protein and in the membrane fraction is preferably 10^3 to 10^8 molecules per cell, more preferably 10^5 to 10^7 molecules per cell. As the amount of expression
5 increases, the ligand binding activity per unit of membrane fraction (specific activity) increases so that not only the highly sensitive screening system can be constructed but also large quantities of samples can be assayed with the same lot.

10 To screen the compounds that alter the binding property between ligands and the protein of the present invention described in ① to ③, for example, an appropriate protein fraction and a labeled ligand are necessary.

15 The protein fraction is preferably a fraction of naturally occurring receptor protein or a recombinant receptor protein fraction having an activity equivalent to that of the natural protein. Herein, the equivalent activity is intended to mean a ligand binding activity,
20 a signal transduction activity or the like that is equivalent to that possessed by naturally occurring proteins.

For the labeled ligand, a labeled ligand and a labeled ligand analogue are used. For example, ligands
25 labeled with [^3H], [^{125}I], [^{14}C], [^{35}S], etc. are used.

Specifically, to screen the compounds that alter the binding property between ligands and the protein of the present invention, first, the protein standard is prepared by suspending cells or cell membrane fraction
30 containing the protein of the present invention in a buffer appropriate for the screening. For the buffer, any buffer that does not interfere with the binding of ligands to the protein is usable and examples of such a buffer are phosphate buffer, Tris-hydrochloride buffer,
35 etc., having pH of 4 to 10 (preferably pH of 6 to 8).

To minimize a non-specific binding, a surfactant such as CHAPS, Tween-80TM (Kao-Atlas Co.), digitonin, deoxycholate, etc. may be added to the buffer. To inhibit degradation of the receptor and ligands by proteases, protease inhibitors such as PMSF, leupeptin, E-64 (manufactured by Peptide Research Laboratory, Co.), and pepstatin may be added. To 0.01 to 10 ml of the receptor solution, a given amount (5,000 to 500,000 cpm) of labeled ligand is added, and 10^{-4} M - 10^{-10} M of a test compound is simultaneously added to be co-present. To examine non-specific binding (NSB), a reaction tube containing an unlabeled test compound in a large excess is also prepared. The reaction is carried out at approximately 0 to 50°C, preferably about 4 to 37°C for about 20 minutes to about 24 hours, preferably about 30 minutes to about 3 hours. After completion of the reaction, the reaction mixture is filtrated through glass fiber filter paper, etc. and washed with an appropriate volume of the same buffer. The residual radioactivity on the glass fiber filter paper is then measured by means of a liquid scintillation counter or γ -counter. Regarding the count obtained by subtracting the amount of non-specific binding (NSB) from the count obtained in the absence of any competitive substance (B_0) as 100%, when the amount of specific binding (B -NSB) is, for example, 50% or less, the test compound can be selected as a candidate substance having a potential of competitive inhibition.

To perform the methods ④ and ⑤ supra of screening the compounds that alter the binding property between ligands and the protein of the present invention, the protein-mediated cell-stimulating activity (e.g., activity that promotes or inhibits arachidonic acid release, acetylcholine release, intracellular Ca^{2+}

release, intracellular cAMP production, intracellular cGMP production, inositol phosphate production, changes in cell membrane potential, phosphorylation of intracellular proteins, activation of c-fos, pH reduction, etc.) can be measured using publicly known methods or commercially available kits.

Specifically, the cells containing the protein of the present invention are first cultured on a multi-well plate, etc. Prior to screening, the medium is replaced with fresh medium or with an appropriate non-cytotoxic buffer, followed by incubation for a given period of time in the presence of a test compound, etc. Subsequently, the cells are extracted or the supernatant is recovered and the resulting product is quantified by appropriate procedures. Where it is difficult to detect the production of the index substance (e.g., arachidonic acid) for the cell-stimulating activity due to a degrading enzyme contained in the cells, an inhibitor against such a degrading enzyme may be added prior to the assay. For detecting activities such as the cAMP production suppression activity, the baseline production in the cells is increased by forskolin or the like and the suppressing effect on the increased baseline production may then be detected.

Screening by assaying the cell-stimulating activity requires cells that have expressed an appropriate protein. For the cells that have expressed the protein of the present invention, the cell line possessing the native protein of the present invention, the cell line expressing the recombinant protein described above and the like are desirable.

For the test compound, for example, peptides, proteins, non-peptide compounds, synthetic compounds, fermentation products, cell extracts, plant extracts,

and animal tissue extracts are used. These compounds may be novel or known compounds.

The kits for screening the compounds or their salts that alter the binding property between ligands and the protein of the present invention comprise the protein of the present invention, cells containing the protein of the present invention, or the membrane fraction of cells containing the protein of the present invention.

Examples of the screening kits of the present invention are as follow.

1. Reagents for screening

① Buffer for measurement and washing

Hanks' balanced salt solution (manufactured by Gibco Co.) supplemented with 0.05% bovine serum albumin (manufactured by Sigma Co.).

The solution is sterilized by filtration through a 0.45 μm filter, and stored at 4°C or may be prepared at use.

② Standard G protein-coupled receptor

CHO cells expressing the protein of the present invention are passaged in a 12-well plate at a density of 5×10^5 cells/well followed by culturing at 37°C under 5% CO₂ and 95% air for 2 days.

③ Labeled ligands

Aqueous solutions of ligands labeled with commercially available [³H], [¹²⁵I], [¹⁴C], [³⁵S], etc. are stored at 4°C or -20°C, and diluted to 1 μM with the measurement buffer.

④ Standard ligand solution

The ligand is dissolved in and adjusted to 1 mM with PBS containing 0.1% bovine serum albumin (manufactured by Sigma Co.) and stored at -20°C.

2. Measurement method

① CHO cells expressing the protein of the present invention are cultured in a 12-well culture plate and washed twice with 1 ml of the measurement buffer, and 490 μ l of the measurement buffer is added to each well.

5 ② After adding 5 μ l of 10^{-3} - 10^{-10} M test compound solution, 5 μ l of a labeled ligand is added to the mixture, and the cells are incubated at room temperature for an hour. To determine the amount of the non-specific binding, 5 μ l of 10^{-3} M non-labeled
10 ligand is added in place of the test compound.

③ The reaction solution is removed, and the wells are washed 3 times with the washing buffer. The labeled ligand bound to the cells is dissolved in 0.2N NaOH-1% SDS, and mixed with 4 ml of liquid scintillator
15 A (manufactured by Wako Pure Chemical Industries, Ltd.)

④ The radioactivity is measured using a liquid scintillation counter (manufactured by Beckman Co.), and the percent maximum binding (PMB) is calculated by the equation below.

20
$$\text{PMB} = [(B - \text{NSB}) / (B_0 - \text{NSB})] \times 100$$

PMB : Percent maximum binding

B : Value obtained in the presence of a test compound

NSB : Non-specific binding

25 B_0 : Maximum binding

The compounds or their salts, which are obtainable using the screening methods or the screening kits of the present invention, are the compounds that alter the
30 binding property between ligands and the protein of the present invention. Specifically, these compounds are:
(a) compounds that have the G protein-coupled receptor-mediated cell-stimulating activity (e.g., activity that promotes or inhibits arachidonic acid release,
35 acetylcholine release, intracellular Ca^{2+} release,

intracellular cAMP production, intracellular cGMP production, inositol phosphate production, changes in cell membrane potential, phosphorylation of intracellular proteins, activation of c-fos, pH reduction, etc.) (so-called agonists to the protein of the present invention); (b) compounds having no cell stimulating-activity (so-called antagonists to the protein of the present invention); (c) compounds that increase the binding affinity between ligands and the G protein-coupled protein of the present invention; and (d) compounds that reduce the binding affinity between ligands and the G protein-coupled protein of the present invention.

The compounds may be peptides, proteins, non-peptide compounds, synthetic compounds, fermentation products, and may be novel or known compounds.

Since agonists to the protein of the present invention have the same physiological activities as those of the ligands for the protein of the present invention, the agonists are useful as safe and low-toxic pharmaceuticals, correspondingly to the ligand activities (prophylactic and/or therapeutic agents for, e.g., central dysfunction (e.g., Alzheimer's disease, senile dementia, suppression of eating (anorexia), epilepsy, etc.), hormone diseases (e.g., weak pains, atonic bleeding, before and after expulsion, subinvolution of uterus, cesarean section, induced abortion, galactostasis, etc.), liver/gallbladder/pancreas/endocrine-associated diseases (e.g., diabetes mellitus, suppression of eating, etc.), inflammatory diseases (e.g., allergy, asthma, rheumatoid, etc.), circulatory diseases (e.g., hypertension, cardiac hypertrophy, angina pectoris, arteriosclerosis, etc.).

Since antagonists to the protein of the present invention can suppress the physiological activities of ligands for the protein of the present invention, the antagonists are useful as safe and low-toxic pharmaceuticals that inhibit the ligand activities (prophylactic and/or therapeutic agents for, e.g., accommondational agents for hormonal secretion, central dysfunction caused of overproducing of ligand to the protein of the present invention, hormone diseases, liver/gallbladder/pancreas/endocrine-associated diseases (e.g., diabetes mellitus, suppression of eating, etc.), inflammatory diseases, circulatory diseases).

The compounds that reduce the binding affinity between ligands and the G protein-coupled receptor protein of the present invention are useful as safe and low-toxic pharmaceuticals that decrease the physiological activities of ligands for the protein of the present invention (prophylactic and/or therapeutic agents for, e.g., accommondational agents for hormonal secretion, central dysfunction caused of overproducing of ligand to the protein of the present invention, hormone diseases, liver/gallbladder/pancreas/endocrine-associated diseases (e.g., diabetes mellitus, suppression of eating, etc.), inflammatory diseases, circulatory diseases).

When compounds or their salt forms, which are obtainable by the screening methods or using the screening kits of the present invention, are employed as ingredients of the pharmaceuticals described above, the compounds can be formulated in the pharmaceuticals in a conventional manner. For example, the compounds can be prepared into tablets, capsules, elixir, microcapsules, aseptic solution, suspension, etc., as

described for pharmaceuticals containing the protein of the present invention.

The preparations thus obtained are safe and low-toxic, and can be administered to, for example,
5 human and mammals (e.g., rats, rabbits, sheep, swine, bovine, cats, dogs, monkeys, etc.).

The dose of the compounds or their salt forms varies depending on subject to be administered, target organs, conditions, routes for administration, etc.; in
10 oral administration, e.g., for the adult patient, the dose is normally about 0.1 mg to about 100 mg, preferably about 1.0 to about 50 mg, and more preferably about 1.0 to about 20 mg per day (as 60 kg body weight). In parenteral administration, the single
15 dose varies depending on subject to be administered, target organ, conditions, routes for administration, etc. but it is advantageous, e.g., for the adult patient, to administer the active ingredient intravenously in a daily dose of about 0.01 to about 30
20 mg, preferably about 0.1 to about 20 mg, and more preferably about 0.1 to about 10 mg (as 60 kg body weight). For other animal species, the corresponding dose as converted per 60 kg body weight can be administered.

25

(6) Quantification of the protein of the present invention, its partial peptide, or its salt form

The antibodies of the present invention are capable of specifically recognizing the protein of the
30 present invention. Therefore, the antibodies can be used to quantify the protein of the present invention in a test fluid, especially for quantification by the sandwich immunoassay. That is, the present invention provides, for example, the following quantification
35 methods:

(i) a method of quantifying the protein of the present invention in a test fluid, which comprises competitively reacting the antibody of the present invention with the test fluid and a labeled form of the protein of the present invention, and measuring the
5 ratio of the labeled protein bound to the antibody; and,

(ii) a method of quantifying the protein of the present invention in a test fluid, which comprises reacting the test fluid with the antibody of the present invention immobilized on a carrier and a
10 labeled form of the antibody of the present invention simultaneously or sequentially, and measuring the activity of the label on the immobilized carrier.

In (ii) described above, it is preferred that one
15 antibody recognizes the N-terminal region of the protein of the present invention, and another antibody reacts with the C-terminal region of the protein of the present invention.

Using monoclonal antibodies to the protein of the present invention (hereinafter sometimes referred to as the monoclonal antibodies of the present invention), the protein of the present invention can be assayed and also detected by tissue staining or the like. For this purpose, an antibody molecule itself may be used, or
25 $F(ab')_2$, Fab' or Fab fractions of the antibody molecule may also be used. Assay methods using antibodies to the protein of the present invention are not particularly limited. Any assay method can be used, so long as the amount of antibody, antigen, or antibody-
30 antigen complex corresponding to the amount of antigen (e.g., the amount of the protein) in the test fluid can be detected by chemical or physical means and the amount of the antigen can be calculated from a standard curve prepared from standard solutions containing known
35 amounts of the antigen. For example, nephrometry,

competitive methods, immunometric method, and sandwich method are appropriately used, with the sandwich method described below being most preferable in terms of sensitivity and specificity.

5 As the labeling agent for the methods using labeled substances, there are employed, for example, radioisotopes, enzymes, fluorescent substances, luminescent substances, etc. For the radioisotope, for example, [^{125}I], [^{131}I], [^3H] and [^{14}C] are used. As the
10 enzyme described above, stable enzymes with high specific activity are preferred; for example, β -galactosidase, β -glucosidase, alkaline phosphatase, peroxidase, malate dehydrogenase and the like are used. Example of the fluorescent substance used are
15 fluorescamine and fluorescein isothiocyanate are used. For the luminescent substance, for example, luminol, luminol derivatives, luciferin, and lucigenin. Furthermore, the biotin-avidin system may be used for binding antibody or antigen to the label.

20 For immobilization of antigen or antibody, physical adsorption may be used. Chemical binding methods conventionally used for insolubilization or immobilization of proteins or enzymes may also be used. For the carrier, for example, insoluble polysaccharides
25 such as agarose, dextran, cellulose, etc.; synthetic resin such as polystyrene, polyacrylamide, silicon, etc., and glass or the like are used.

 In the sandwich method, the immobilized monoclonal antibody of the present invention is reacted with a
30 test fluid (primary reaction), then with the labeled monoclonal antibody of the present invention (secondary reaction), and the activity of the label on the immobilizing carrier is measured, whereby the amount of the protein of the present invention in the test fluid
35 can be quantified. The order of the primary and

secondary reactions may be reversed, and the reactions may be performed simultaneously or with an interval. The methods of labeling and immobilization can be performed by the methods described above.

5 In the immunoassay by the sandwich method, the antibody used for immobilized or labeled antibodies is not necessarily one species, but a mixture of two or more species of antibody may be used to increase the measurement sensitivity.

10 In the methods of assaying the protein of the present invention by the sandwich method, antibodies that bind to different sites of the protein are preferably used as the monoclonal antibodies of the present invention for the primary and secondary
15 reactions. That is, in the antibodies used for the primary and secondary reactions are, for example, when the antibody used in the secondary reaction recognizes the C-terminal region of the protein, it is preferable to use the antibody recognizing the region other than
20 the C-terminal region for the primary reaction, e.g., the antibody recognizing the N-terminal region.

 The monoclonal antibodies of the present invention can be used for the assay systems other than the sandwich method, for example, competitive method,
25 immunometric method, nephrometry, etc. In the competitive method, antigen in a test fluid and the labeled antigen are competitively reacted with antibody, and the unreacted labeled antigen (F) and the labeled antigen bound to the antibody (B) are separated (B/F
30 separation). The amount of the label in B or F is measured, and the amount of the antigen in the test fluid is quantified. This reaction method includes a liquid phase method using a soluble antibody as an antibody, polyethylene glycol for B/F separation and a
35 secondary antibody to the soluble antibody, and an

immobilized method either using an immobilized antibody as the primary antibody, or using a soluble antibody as the primary antibody and immobilized antibody as the secondary antibody.

5 In the immunometric method, antigen in a test fluid and immobilized antigen are competitively reacted with a definite amount of labeled antibody, the immobilized phase is separated from the liquid phase, or antigen in a test fluid and an excess amount of
10 labeled antibody are reacted, immobilized antigen is then added to bind the unreacted labeled antibody to the immobilized phase, and the immobilized phase is separated from the liquid phase. Then, the amount of the label in either phase is measured to quantify the
15 antigen in the test fluid.

 In the nephrometry, insoluble precipitate produced after the antigen-antibody reaction in gel or solution is quantified. When the amount of antigen in the test fluid is small and only a small amount of precipitate
20 is obtained, laser nephrometry using scattering of laser is advantageously employed.

 For applying these immunological methods to the measurement methods of the present invention, any particular conditions or procedures are not required.
25 Systems for measuring the protein of the present invention or its salts are constructed by adding the usual technical consideration in the art to the conventional conditions and procedures. For the details of these general technical means, reference can
30 be made to the following reviews and texts. [For example, Hiroshi Irie, ed. "Radioimmunoassay" (Kodansha, published in 1974), Hiroshi Irie, ed. "Sequel to the Radioimmunoassay" (Kodansha, published in 1979), Eiji Ishikawa, et al. ed. "Enzyme immunoassay" (Igakushoin,
35 published in 1978), Eiji Ishikawa, et al. ed.

"Immunoenzyme assay" (2nd ed.) (Igakushoin, published in 1982), Eiji Ishikawa, et al. ed. "Immunoenzyme assay" (3rd ed.) (Igakushoin, published in 1987), Methods in ENZYMOLOGY, Vol. 70 (Immunochemical Techniques (Part A)), *ibid.*, Vol. 73 (Immunochemical Techniques (Part B)), *ibid.*, Vol. 74 (Immunochemical Techniques (Part C)), *ibid.*, Vol. 84 (Immunochemical Techniques (Part D: Selected Immunoassays)), *ibid.*, Vol. 92 (Immunochemical Techniques (Part E: Monoclonal Antibodies and General Immunoassay Methods)), *ibid.*, Vol. 121 (Immunochemical Techniques (Part I: Hybridoma Technology and Monoclonal Antibodies)) (all published by Academic Press Publishing).

As described above, the protein of the present invention or its salts can be quantified with high sensitivity, using the antibodies of the present invention. By quantifying the protein of the present invention or its salts using the antibodies of the present invention, diagnosis can be made on various diseases.

The antibodies of the present invention can also be used for specifically detecting the protein of the present invention present in test samples such as body fluids or tissues. The antibodies may also be used for preparation of antibody columns for purification of the protein of the present invention, for detection of the protein of the present invention in each fraction upon purification, and for analysis of the behavior of the protein of the present invention in the test cells.

(7) Preparation of non-human animals carrying the DNA encoding the G protein-coupled receptor protein of the present invention

Using the DNA of the present invention, non-human transgenic animals expressing the protein of the

present invention can be prepared. Examples of the non-human animals include mammals (e.g., rats, mice, rabbits, sheep, swine, bovine, cats, dogs, monkeys, etc.) (hereinafter merely referred to as animals) can
5 be used, with mice and rabbits being particularly appropriate.

To transfer the DNA of the present invention to target animals, it is generally advantageous to use the DNA in a gene construct ligated downstream of a
10 promoter that can express the DNA in animal cells. For example, when the DNA of the present invention derived from rabbit is transferred, e.g., the gene construct, in which the DNA is ligated downstream of a promoter that can express the DNA of the present invention
15 derived from animals containing the DNA of the present invention highly homologous to the rabbit-derived DNA, is microinjected to rabbit fertilized ova; thus, the DNA-transferred animal, which is capable of producing a high level of the protein of the present invention, can
20 be produced. Examples of the promoters that are usable include virus-derived promoters and ubiquitous expression promoters such as metallothionein promoter, but promoters of NGF gene and enolase that are specifically expressed in the brain are preferably used.

25 The transfer of the DNA of the present invention at the fertilized egg cell stage secures the presence of the DNA in all germ and somatic cells in the produced animal. The presence of the protein of the present invention in the germ cells in the DNA-
30 transferred animal means that all germ and somatic cells contain the protein of the present invention in all progenies of the animal. The progenies of the animal that took over the gene contain the protein of the present invention in all germ and somatic cells.

The DNA-transferred animals of the present invention can be maintained and bred in the conventional environment as animals carrying the DNA after confirming the stable retention of the gene in the animals through mating. Furthermore, mating male and female animals containing the objective DNA results in acquiring homozygote animals having the transferred gene on both homologous chromosomes. By mating the male and female homozygotes, breeding can be performed so that all progenies contain the DNA.

Since the protein of the present invention is highly expressed in the animals in which the DNA of the present invention has been transferred, the animals are useful for screening of agonists or antagonists to the protein of the present invention.

The animals in which the DNA of the present invention has been transferred can also be used as cell sources for tissue culture. The protein of the present invention can be analyzed by, for example, directly analyzing the DNA or RNA in tissues from the mouse in which the DNA of the present invention has been transferred, or by analyzing tissues containing the protein expressed from the gene. Cells from tissues containing the protein of the present invention are cultured by the standard tissue culture technique. Using these cells, for example, the function of tissue cells such as cells derived from the brain or peripheral tissues, which are generally difficult to culture, can be studied. Using these cells, for example, it is possible to select pharmaceuticals that increase various tissue functions. When a highly expressing cell line is available, the protein of the present invention can be isolated and purified from the cell line.

In the specification and drawings, the codes of bases and amino acids are denoted in accordance with the IUPAC-IUB Commission on Biochemical Nomenclature or by the common codes in the art, examples of which are shown below. For amino acids that may have the optical isomer, L form is presented unless otherwise indicated.

DNA : deoxyribonucleic acid
cDNA : complementary deoxyribonucleic acid
A : adenine
10 T : thymine
G : guanine
C : cytosine
RNA : ribonucleic acid
mRNA : messenger ribonucleic acid
15 dATP : deoxyadenosine triphosphate
dTTP : deoxythymidine triphosphate
dGTP : deoxyguanosine triphosphate
dCTP : deoxycytidine triphosphate
ATP : Adenosine triphosphate
20 EDTA : ethylenediamine tetraacetic acid
SDS : sodium dodecyl sulfate
Gly: glycine
Ala: alanine
Val: valine
25 Leu: leucine
Ile: isoleucine
Ser: serine
Thr: threonine
Cys: cysteine
30 Met: methionineGlu : glutamic acid
Asp : aspartic acid
Lys : lysine
Arg : arginine
His : histidine
35 Phe : phenylalanine

Tyr : tyrosine
 Trp : tryptophan
 Pro : proline
 Asn : asparagine
 5 Gln : glutamine
 pGlu : pyroglutamic acid
 Tos : p-toluenesulfonyl
 CHO : formyl
 Bzl : benzyl
 10 Cl₂Bzl: 2,6-dichlorobenzyl
 Bom : benzyloxymethyl
 Z : benzyloxycarbonyl
 Cl-Z : 2-chlorobenzyloxycarbonyl
 Br-Z : 2-bromobenzyloxycarbonyl
 15 Boc : t-butoxycarbonyl
 DNP : dinitrophenol
 Trt : trityl
 Bum : t-butoxymethyl
 Fmoc : N-9-fluorenylmethoxycarbonyl
 20 HOBt : 1-hydroxybenztriazole
 HOObt: 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-
 benzotriazine
 HONB : 1-hydroxy-5-norbornene-2,3-dicarboximide
 DCC : N,N'-dicyclohexylcarbodiimide

25

The sequence identification numbers in the sequence listing of the specification indicates the following sequence, respectively.

[SEQ ID NO:1]

30

This shows the amino acid sequence of human brain-derived protein of the present invention.

[SEQ ID NO:2]

This shows the base sequence of cDNA encoding human brain-derived protein of the present invention,

which has the amino acid sequence shown by SEQ ID
NO:1(AQ27).

[SEQ ID NO:3]

5 This shows the base sequence of primer 1 used in
Examples 1 and 2.

[SEQ ID NO:4]

This shows the base sequence of primer 2 used in
Examples 1 and 2.

[SEQ ID NO:5]

10 This shows the base sequence of the forward primer
used in Example 2.

[SEQ ID NO:6]

This shows the base sequence of the reverse primer
used in Example 2.

15 [SEQ ID NO:7]

This shows the base sequence of probe used in
Example 2.

20 *Escherichia coli* DH5 α /pCDNA3.1-AQ27 obtained in
Example 1 later described was on deposit with the
Ministry of International Trade and Industry, Agency of
Industrial Science and Technology, National Institute
of Bioscience and Human Technology (NIBH), located at
1-1-3, Higashi, Tsukuba-shi, Ibaraki, Japan, as the
25 Accession Number FERM BP-6854 on August 23, 1999 and
with Institute for Fermentation, Osaka (IFO), located
at 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka-shi,
Osaka, Japan, as the Accession Number IFO 16304 on
August 4, 1999.

30

EXAMPLES

The present invention is described in detail below
with reference to EXAMPLES, which are not deemed to
limit the scope of the present invention. The gene
35 manipulation procedures using *Escherichia coli* were

performed according to the methods described in the Molecular Cloning.

EXAMPLE 1: Cloning of the cDNA encoding the G protein-coupled receptor protein (AQ27) and determination of the base sequence

Using human fetal brain cDNA (CLONTECH Inc.) as a template and two primers, namely, primer 1 (5'-TGT CAG CAT GCA GGC GCT TAA CAT TAC CCC GGA GCA G -3'; SEQ ID NO:3) and primer 2 (5'-GAC TAG TTT AAT GCC CAC TGT CTA AAG GAG AAT TCT C -3'; SEQ ID NO:4), a PCR reaction was carried out. The reaction solution in the above reaction comprised 1/10 volume of the cDNA for the template, 1/50 volume of Advantage 2 Polymerase Mix (CLONTECH Inc.), 0.2 μ M of primer 1, 0.2 μ M of primer 2, 200 μ M of dNTPs and a buffer attached to the enzyme to make the final volume 25 μ l. In the PCR reaction, after (1) heating the reaction solution at 95°C for 1 minute, (2) a cycle of heating at 95°C for 30 seconds followed by 72°C for 4 minutes, was repeated 5 times, (3) a cycle of heating at 95°C for 30 seconds followed by 70°C for 4 minutes, was repeated 5 times, (4) a cycle of heating at 95°C for 30 seconds followed by 68°C for 30 seconds and 66°C for 4 minutes, was repeated 30 times, and (3) finally, an extension reaction was carried out at 68°C for 3 minutes. After completion of the PCR reaction, the reaction product was subcloned to plasmid vector pCDNA3.1 /V5/His (Invitrogen Inc.) according to the instructions attached to the TA cloning kit (Invitrogen Inc.), which was named pCDNA3.1-AQ27. Then, it was introduced into Escherichia coli DH5 α , and the clones containing the cDNA were selected on LB agar plates containing ampicillin. The sequence of each clone was analyzed to give the cDNA sequence encoding the novel G protein-coupled receptor

protein. The novel G protein-coupled receptor protein containing the amino acid sequence deduced therefrom was designated AQ27 and the transformant was designated Escherichia coli DH5 α /pcDNA3.1-AQ27

5

Example 2: Analysis on distribution of expression of AQ27 in various tissues by TaqMan PCR

First, as primers and a probe, forward primer AQ27TaqF (5'-CAATG CTAGG TGTGG TCTGG CT -3' SEQ ID (NO:5)), reverse primer AQ27TaqR (5'-GATCT CAAGT TGTTG CACGT GC-3' (SEQ ID NO:6)) and probe AQ27-515T (5'-TGGCA GTCAT CGTAG GATCA CCCAT G-3' (SEQ ID NO:7)) were designed using Primer Express Ver.1.0 (PE Biosystems Japan). FAM (6-carboxyfluorescein) was added as a reporter dye.

Standard cDNA was prepared by following: The PCR fragment was amplified using pcDNA3.1-AQ27 as a template, and Primer 1 (SEQ ID NO:3) and Primer 2 (SEQ ID NO:4), purified with PCR purification Kit (QIAGEN, Germany), and then adjusted to make a final concentration of 10^0 - 10^6 copies/ μ l at use.

Human Tissue cDNA Panel I and Panel II (CLONTECH Laboratories, Inc., CA, USA) were used as a cDNA source of each tissue. A TaqMan PCR reaction was carried out using Universal PCR Master Mix as a reagent with ABI PRISM 7700 Sequence Detection System (PE Biosystems Japan) according to the directions attached thereto.

The results are shown in Figure 4 and Table 1. AQ27 shows high expression in the heart, liver and testis.

30

Table 1

<u>Tissue</u>	<u>Expression (copies/μl)</u>
brain	39
heart	158
kidney	74
liver	0
lung	1
pancreas	2
placenta	2
skeltal muscle	4
colon	17
ovary	2
leukocyte	25
prostate	32
small intestine	16
spleen	2
testis	50
thymus	6

5

Example 3: Reactivity of AQ27 with respect to Met-Enkephalin-Arg-Phe amide

The AQ27-expressing CHO cells and mock cells were inoculated in a capsule for Cytosensor at a concentration of 2.7×10^5 cells/capsule, respectively, incuvated overnight, and mounted onto Cytosensor(Molecular Device). After the acidification rare became stable, the cells were exposed to 10 μ M Enkephalin-Arg-Phe amide (Bachem, H-2835) for 7 minutes and 2 seconds. The cell activation was measured using the increasing rate of the acidification rate as index. As a result, it was confirmed that Met-Enkephalin-Arg-Phe amide specifically activates the AQ27-expressing

CHO cells and it is specifically reacted with AQ27
(Figure 5).

INDUSTRIAL APPLICABILITY

5 The protein of the present invention, its partial
peptides, or salts thereof and the DNA encoding the
same can be used for; ① determination of ligands
(agonists); ② preparation of antibodies and antisera;
③ construction of recombinant protein expression
10 systems; ④ development of the receptor binding assay
systems using the expression systems and screening of
pharmaceutical candidate compounds; ⑤ effecting drug
design based on comparison with structurally similar
ligand receptors; ⑥ reagents for preparation of probes
15 and PCR primers for gene diagnosis; ⑦ production of
transgenic animals; and ⑧ pharmaceutical drugs for
the gene prophylaxis/therapy.

CLAIMS

1. A protein which comprises the same or
5 substantially the same amino acid sequence as the amino
acid sequence represented by SEQ ID NO:1, or a salt
thereof.
2. A partial peptide of the protein according to
claim 1, or a salt thereof.
- 10 3. A DNA which comprises a DNA encoding the
protein according to claim 1.
4. A DNA according to claim 3, which has the base
sequence represented by SEQ ID NO:2.
5. A recombinant vector which comprises the DNA
15 according to claim 3.
6. A transformant transformed with the recombinant
vector according to claim 5.
7. A method of producing the protein or its salt
according to claim 1, which comprises culturing the
20 transformant according to claim 6 and producing and
accumulating the protein according to claim 1.
8. An antibody to the protein according to claim 1,
the partial peptide according to claim 2, or a salt
thereof.
- 25 9. A method of determining a ligand to the protein
or its salt according to claim 1, which comprises using
the protein according to claim 1 or the partial peptide
according to claim 2, or a salt thereof.
10. A method of screening a compound or its salt
30 that alters the binding property between a ligand and
the protein or its salt according to claim 1, which
comprises using the protein according to claim 1, the
partial peptide according to claim 2, or a salt thereof.
11. A kit for screening a compound or its salt
35 that alters the binding property between a ligand and

the protein or its salt according to claim 1, which comprises the protein according to claim 1 or the partial peptide according to claim 2, or a salt thereof.

12. A compound or its salt that alters the binding
5 property between a ligand and the protein or its salt according to claim 1, which is obtainable using the screening method according to claim 10 or the screening kit according to claim 11.

13. A pharmaceutical composition which comprises a
10 compound or its salt that alters the binding property between a ligand and the protein or its salt according to claim 1, which is obtainable using the screening method according to claim 10 or the screening kit according to claim 11.

14. A DNA which hybridizes to the DNA according to
15 claim 3 under highly stringent conditions.

ABSTRACT OF THE DISCLOSURE

The present invention relates to a human-derived protein, its partial peptides, or salts thereof,
5 methods for determining a ligand to the protein, screening methods/screening kits for a compound that alters the binding property between a ligand and the protein, a compound obtainable by the screening or its salts, etc.

10 The human-derived protein of this invention or the DNA encoding the protein can be used for ① determination of ligands to the the present invention; ② prophylactic and/or therapeutic agents for diseases associated with dysfunction of the protein of the
15 present invention; ③ screening of compounds (agonists, antagonists, etc.) that alter the binding property between the protein of the present invention and ligands.

Fig. 1

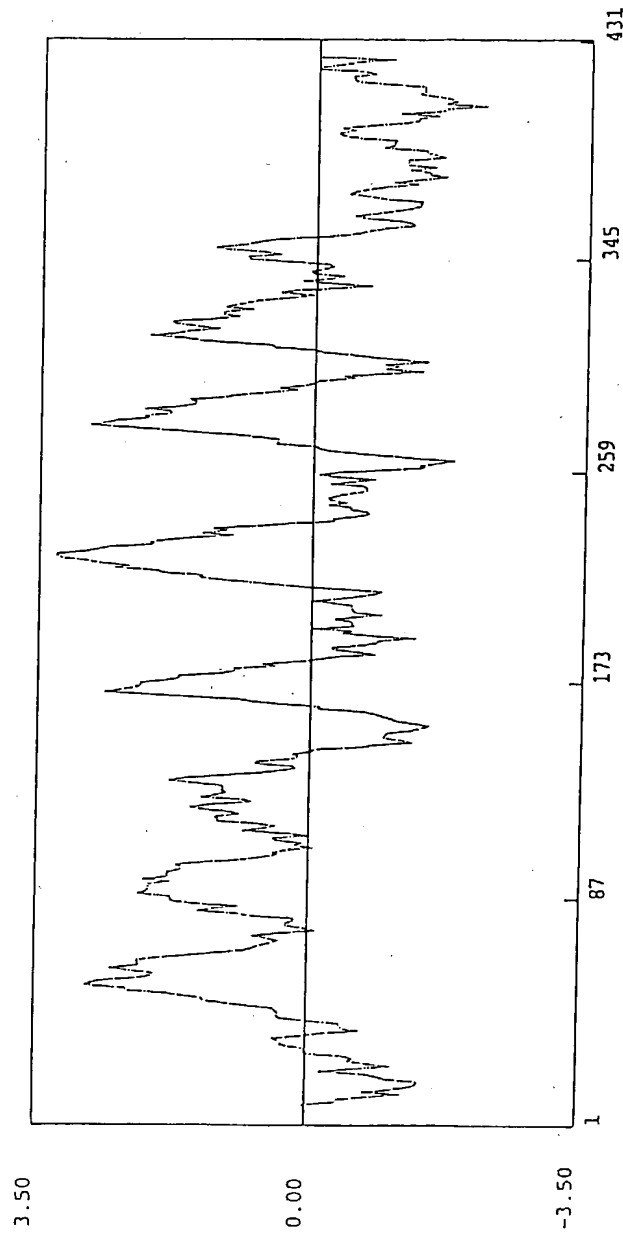
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370	380	390	400	410	420	430	440	450	460	470	480
490	500	510	520	530	540	550	560	570	580	590	600
610	620	630	640	650	660	670	680	690	700	710	720
730	740	750	760	770	780	790	800	810	820	830	840
850	860	870	880	890	900	910	920	930	940	950	960
970	980	990	1000	1010	1020	1030	1040	1050	1060	1070	1080
1090	1100	1110	1120	1130	1140	1150	1160	1170	1180	1190	1200
1210	1220	1230	1240	1250	1260	1270	1280	1290	1300	1310	1320
1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430	1440
1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560

Fig. 2

1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680
AGACAGAGGAGAGAAAAAGCTCAAACGACATCTTGCTCTCTTTAGTGCTGAAGTTCCTTTAGACAGTGGGCATTTAATTATAACAATATCTTCAATAATTAAATGCGCTT
T E E K K K L K R H L A L F R S E L A E N S P L D S G H .
1690 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
CAGATTGTAAACCCAAAGAGAAAATTATTTTGGAGCAAGGTCAATAATCTTTTAATTCTTAAGATGACAGAAGAAAACAATCATGTGTTTCCATTAAAAAATGACACGAGGCTAGTC
1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
CAAGTCAGTGAATGTTTACAAACCAATNGATCACAATCATTTAACANATTTCTGTGTTCTTCTCATTCCTGCTTCCACTGCTTCACTTGAAGTTCCTTAAAAAGCAACATGGAAGGCCAGGCAGC
1930 1940 1950 1960 1970 1980 1990 2000 2010 2020 2030 2040
GTGGCTCATGCTGTAAATCCAGCACTTTGGGAGGCTAGACGGCGGATCAGAGTCAAGAGATCAAAACCATCTCGGCTAACAGGGTGAACCCCATCTCTGCTTAAAAAATACAAATA
2050 2060 2070 2080 2090 2100 2110 2120 2130 2140 2150 2160
TTAGCCGGCGGTGGTGGCGGGCACTGTAGTCCAGCTACTTGGAGGCTCAGCGGAGAAATGTTGTAACCCGGAGGCGGAGCTTGCAGTGATCCGAGATCATGCCACTGCCTCCA
2170 2180 2190 2200 2210 2220 2230 2240 2250 2260 2270 2280
GCCTGGCGGAAAGACGAGACTCCCGCTCAAAAAAATTTTTTTGAAAAATTCGTAACCATACTTTTAAGATTATTTCAAGTGGATTTTTTAAAAATCTTGTCAGAAATCAGGGTTCT
2290 2300 2310 2320 2330 2340 2350 2360 2370 2380 2390 2400
TAGCTAGCAGTTTTTCTCCACCGAGTCACTGTAATGTGACTATGTATCTAGATTGAATAGAAAAATAAATAATATCTTCTTCTGNAAAAAAATAAAAAAATAAAAAA
2410 2420
AAAAAAAAAAAAAAGG

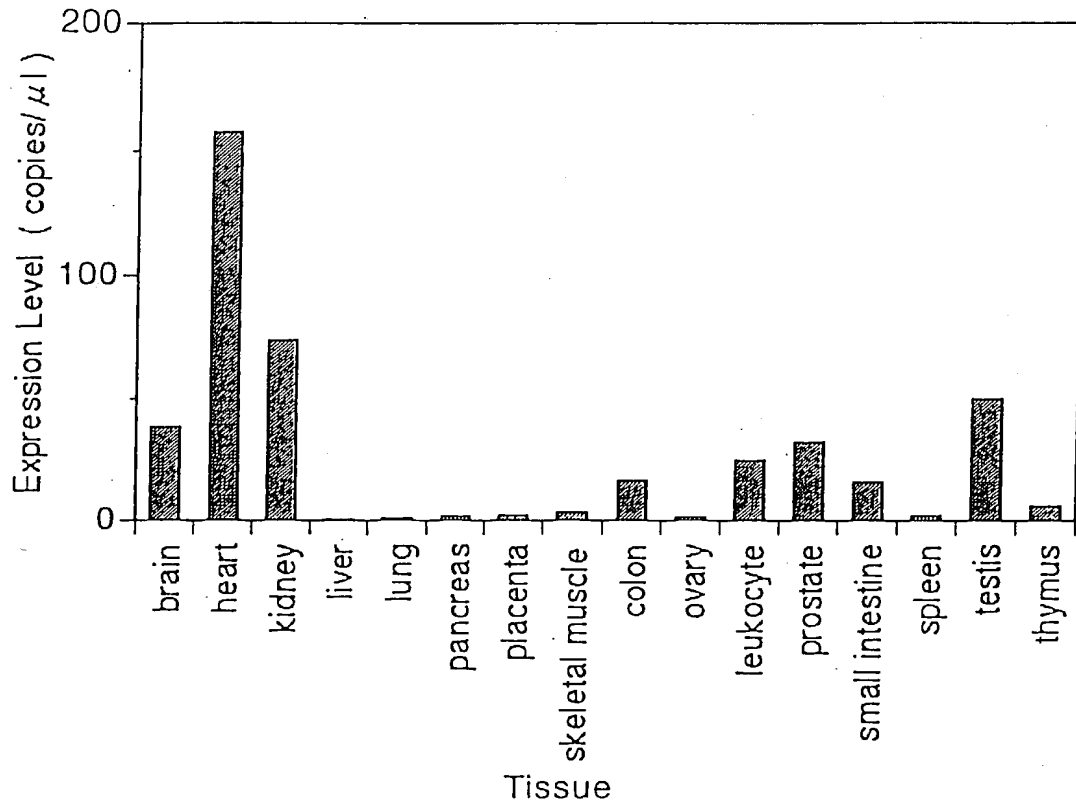
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Fig.3



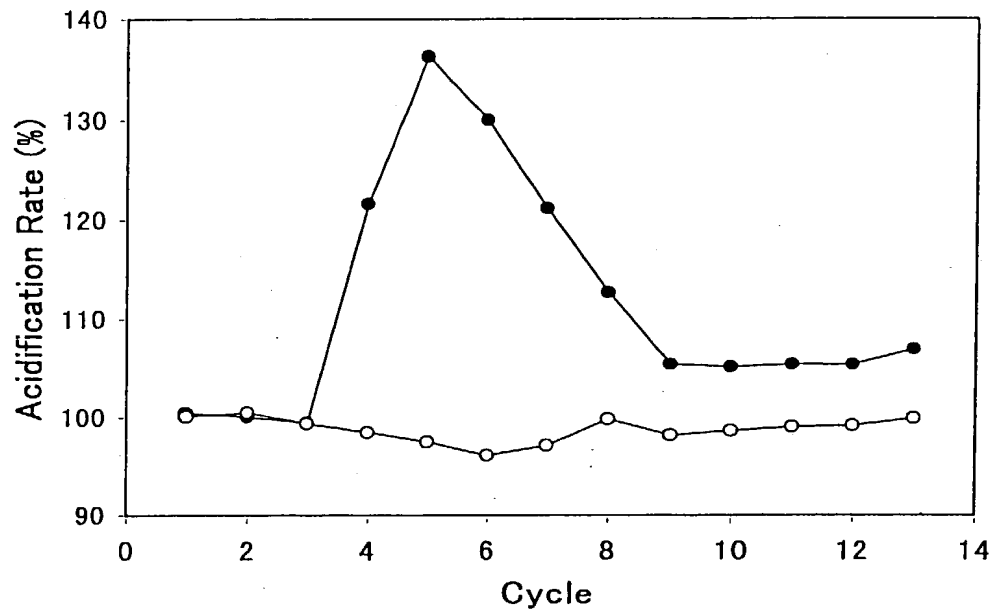
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Fig.4



5/5

Fig.5



Express Mail No: EL933048915US
Attorney Docket No. 57132 (46342)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: T. Watanabe, et al. EXAMINER: Not Yet Assigned
U.S.S.N.: based on PCT/JP00/05684 GROUP: Not Yet Assigned
FILED: February 27, 2002
FOR: NOVEL G PROTEIN-COUPLED RECEPTOR PROTEIN AND DNA THEREOF

Commissioner for Patents
Washington, D.C. 20231

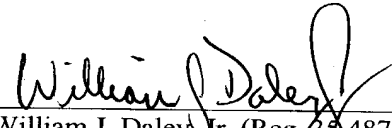
.....
Sir:

ASSOCIATE POWER OF ATTORNEY (37 CFR 1.34)

Please recognize the following as an Associate Agent in this case:
Dianne Rees, Reg. No. 45,281.

Respectfully submitted,

Date: February 27, 2002


William J. Daley, Jr. (Reg. 35,487)
Dike, Bronstein, Roberts & Cushman
Intellectual Property Practice Group of
EDWARDS & ANGELL, LLP
PO BOX 9169
Boston, Massachusetts 02209
Telephone: 1-617-439-4444
Fax: 1-617-439-4170

Customer No: 21,874

BOS2_192250.1

DIKE, BRONSTEIN, ROBERTS & CUSHMAN, LLP
130 Water Street
Boston, Massachusetts 02109

3633050P
Docket No. 57132
246342
Page 1 of 4

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that: My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed at 201) below or an original, first and joint inventor (if plural names are listed at 201-208 below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Novel G Protein-coupled Receptor Protein and DNA Thereof

which is described and claimed in:

☐ the specification attached hereto.

☐ the specification in U.S. Application Serial Number _____, filed on _____

☒ the specification in PCT international application Number PCT/JP00/05684, filed on August 24, 2000; and was amended on _____.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a). I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign/PCT Applications and Any Priority Claims Under 35 U.S.C. §119:			
Application No.	Filing Date	Country	Priority Claimed Under 35 U.S.C. §119?
241530/1999	August 27, 1999	JP	x
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose material information as defined in 37 CFR §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Prior U.S. Applications or PCT International Applications Designating the U.S.-Benefit Under 35 U.S.C. §120					
U.S. Applications			Status (Check One)		
Application Serial No.	U.S. Filing Date		Patented	Pending	Abandoned
PCT Applications Designating the U.S.					
Application No.	Filing Date	U.S. Serial No. Assigned			

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
(35 U.S.C. §119(e))

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Applicant	Provisional Application Number	Filing Date

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) with full powers of association, substitution and revocation to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Sewall P. Bronstein (Reg. No. 16,919)	Peter F. Corless (Reg. No. 33,860)	Richard E. Gamache (Reg. No. 39,196)
David G. Conlin (Reg. No. 27,026)	Cara Z. Lowen (Reg. No. 38,227)	David A. Tucker (Reg. No. 27,840)
George W. Neuner (Reg. No. 26,964)	William J. Daley, Jr. (Reg. No. 35,487)	Lisa Hazzard Swiszc (Reg. No. 44,368)
Linda M. Buckley (Reg. No. 31,003)	Robert L. Buchanan (Reg. No. 40,927)	
Peter J. Manus (Reg. No. 26,766)	Christine C. O'Day (Reg. No. 38,256)	

SEND CORRESPONDENCE TO: David G. Conlin Dike, Bronstein, Roberts & Cushman, LLP 130 Water Street Boston, Massachusetts 02109	DIRECT TELEPHONE CALLS TO: (617) 523-3400
---	---

- 3 -

2 0 1	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	1 - 00	<u>Watanabe</u>	<u>Takuya</u>	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
		<u>Osaka</u> <u>J P X</u>	<u>Japan</u>	<u>Japan</u>
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 14-9-B904, Niitaka 6-chome, Yodogawa-ku, Osaka-shi, Osaka 532-0033 Japan		

2 0 2	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	2 - 00	<u>Kikuchi</u>	<u>Kuniko</u>	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
		<u>Ibaraki</u> <u>J P X</u>	<u>Japan</u>	<u>Japan</u>
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 8-18-101, Shinmachi 5-chome, Toride-shi, Ibaraki 302-0024 Japan		


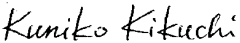

2 0 3	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	3 - 00	<u>Shintani</u>	<u>Yasushi</u>	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
		<u>Osaka</u> <u>J P X</u>	<u>Japan</u>	<u>Japan</u>
	POST OFFICE ADDRESS	POST OFFICE ADDRESS 14-8-606, Niitaka 6-chome, Yodogawa-ku, Osaka-shi, Osaka 532-0033 Japan		

2 0 4	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS		

2 0 5	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS		

- 4 -

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signature of Inventor 201 Takuya Watanabe 	Date: Dec. 13, 2001
Signature of Inventor 202 Kuniko Kikuchi 	Date: Dec. 12, 2001
Signature of Inventor 203 Yasushi Shintani 	Date: Dec. 14, 2001
Signature of Inventor 204	Date:
Signature of Inventor 205	Date:

SEQUENCE LISTING

<110> Takeda Chemical Industries, Ltd.

<120> Novel G Protein Coupled Receptor Protein and Its Use

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<151> 1999-08-27

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<110> WATANABE, TAKUYA
KIKUCHI, KUNIKO
SHINTANI, YASUSHI

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AND DNA THEREOF

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<150> JP 11-241530

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ALL INFORMATION CONTAINED HEREIN IS UNCLASSIFIED
DATE 08-22-2001 BY 60322 UCBAW

<110> Takeda Chemical Industries, Ltd.
<120> Novel G Protein Coupled Receptor Protein and Its Use
<130> 2633W00P
<150> JP 11-241530
<151> 1999-08-27
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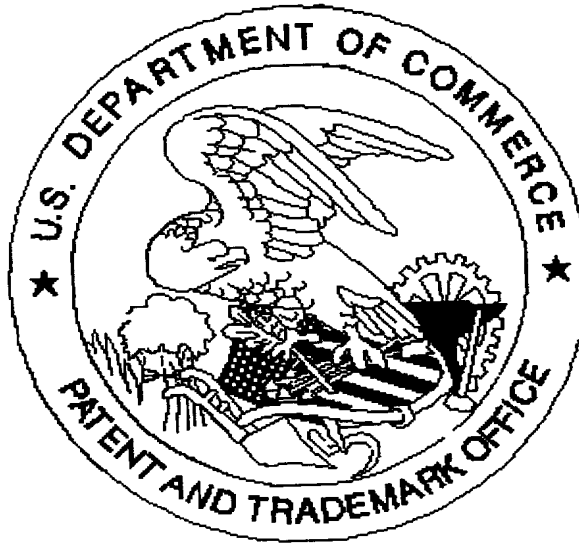
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specification are sequence listing*